The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

The Behavior of Rods in Photopic Vision

By

Toshihiko Okiawa and **Toshio Kurosawa**
(及川 俊彦) (黒澤 敏男)

From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai

(Received for publication, July 27, 1953)

One of the most important problems in vision of to-day is the question as to what role the rods play in photopic vision. Hecht[1] studied the dependence of various visual functions upon the intensity of light and elucidated that the change-over from preponderant rod-activity in dark adaptation to preponderant cone-activity in light adaptation occurs. However, the question raised above was an open one in his investigation. Some people[2-4] believed that the rods play some part in photopic vision, and recently Willmer[5-10] presented a hypothesis that the rods would be active in association with cones, playing a subsidiary role in photopic vision. Brooke[11] and Oikawa[12] showed that the activity of the rods persists even at higher brightness levels than has hitherto been assumed by some workers.

On the other hand, there was some author[13] advocating that the rods are only slightly active, if any, at higher intensities. More recently, Oikawa and Kurosawa[14] have shown some evidence that the rod-activity is suppressed at higher intensities, but none of their data provides evidence for the complete inactivity of the rods at higher brightness levels. Wright[15] suggested that the total suppression of the rod-activity will not take place under usual photopic condition of the retina.

In the present experiment, we studied the behavior of rods and cones by the method of electrostimulation with special reference to the intensity of light in order to clarify the obscurity concerning the role played by rods in photopic vision.

Experimental

Method

The electrical supernormality of the eye after exposure to light is taken as a measure for the effect of the light used.

After a preliminary dark adaptation for about 20 minutes, the eye

HEALTH CENTER LIBRARY

was exposed to white light for 40 msec. and was stimulated with a constant current pulse of 100 msec. in duration at different times after termination of the light stimulus. The index of excitation was the least perceptible electrical phosphene. A pair of silver electrodes were placed on the forehead slightly above the eyebrow and on the homolateral temple.

The effect of an illumination upon the dark-adapted eye was expressed as a percentage increase of the electrical excitability over the resting level and was denoted by ζ.

An opaque glass disc of 0.5 cm. in diameter was illuminated from behind by white light and viewed from a distance of 30 cm., so that the visual angle subtended by the disc at the eye was 1°. In order to illuminate various retinal locations, a fixation mark, a faint red light was displaced along the horizontal meridian of the nasal field of the eye.

The details of the apparatus and procedure to determine electrical excitability are given in Motokawa's papers[16-17].

Results

The ζ-time curves, or the excitability curves for white light of various intensities are illustrated in Fig. 1. In these experiments a flash of white light of 40 msec. in duration was given to a retinal region of 20° from the center of the fovea. The intensities of light used were 300, 50, 10 lux and of threshold strength from the top downwards. We can distinguish three elevations denoted by Y, B and ROD in Fig. 1d. The elevation labeled ROD has its maximum at 4.5 seconds after the onset of the flash and represents the rod-process for the reasons stated by Motokawa *et al.*[18-20] The maxima of the two former elevations lie at 1.5 and 3 seconds from the termination of the light stimulus respectively. Based upon Motokawa's analysis,[16)21)] it can be said that the elevation at 1.5 sec. represents the yellow process (Y) while that at 3 sec. represents the blue process (B).

The curve for a flash of 10 lux in Fig. 1c also consisted of similar three components, Y-, B- and rod-processes. The magnitude of each process is much higher than that of the corresponding process in Fig. 1d. When the intensity of pre-illumination was increased to 50 lux, no rod-process appeared in the excitablity curve, as is shown in Fig. 1b. The total suppression of the rod-activity can be seen also in the curve of Fig. 1a obtained with a flash of 300 lux. It is to be noted that in Fig. 1a not only the Y- and B- processes but also other cone-processes are found; the crest times of these processes are about 1 and 2 seconds respectively, so they are denoted by R (red) and G (green) in the figure.[16)21)]

It deserves attention that no rod-process could be obtained at the periphery 20° from the fovea where the population density of rods is great-

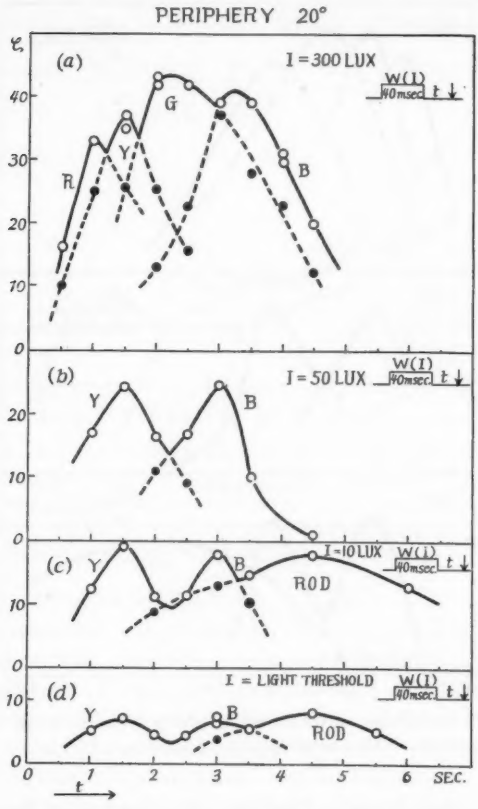


Fig. 1. Excitability curves obtained at periphery 20° with pre-illumination of various intensities. Light-stimulus used was a white flash of 40 msec. Ordinates: Percentage increases of electrical excitability over resting level. Abscissas: Time in sec. after the end of pre-illumination. Experimental procedure is shown in insets. W: White light. Arrow: Electric stimulus.

est in the retina.

Similar experiments were carried out at various retinal locations, the intensity of light being fixed at 50 lux. The results are illustrated in Fig. 2. In this figure it can be seen that the rod-activity is completely depressed at 5° and 20°, whereas a conspicuous rod-process occurs at 50°. The density of rods is lower at 50° than at 20°. Nevertheless, no indication of depression of rod-activity can be found at 50°. This finding suggests that the depression of rod-activity depends not so much upon the density

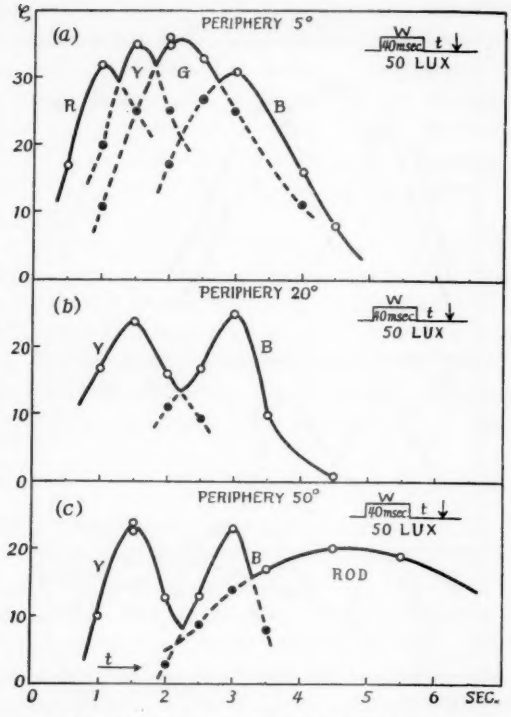


Fig. 2. Excitability curves for white light of 50 lux obtained at various parts of the retina. Other notations are the same as in Fig. 1.

of rods as upon the density of cones; the absence of depression in the extreme periphery of the retina is probably due to a low population of cones. In other words, the depression of rod-activity observed above must have been caused by interaction between cones and rods.

This interpretation will receive further support from following experiments. In Fig. 3 the magnitudes of rod-reponses are represented as a function of intensities of white light. In this figure, the magnitudes of Y-process are presented as a representative of the cone-processes. The full curve connecting empty triangles and the broken curve connecting solid triangles show respectively the magnitudes of the Y- and rod-processes obtained at 5° from the fovea. The full curve marked by empty circles (Y) and the broken curve marked by solid circles (Rod) were obtained at 20°. The curves connecting squares refer to the data obtained at 50°. The arrows indicate the intensities for light threshold determinep

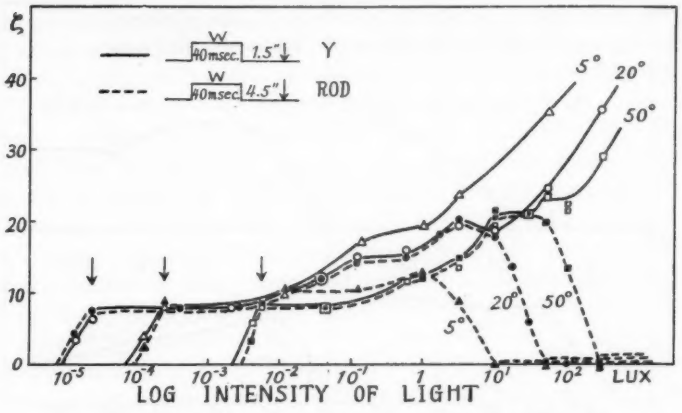


Fig. 3. The dependence of magnitudes of Y- (full line) and rod-processes (broken line) upon logarithm of light intensity. Experimental procedure is shown in inset. The curves were obtained at 5°, 20° and 50° from the fovea. Three arrows indicate threshold intensities determined at 20°, 5° and 50° from the fovea in order from left to right.

at 20°, 5° and 50° in order from left to right.

Generally speaking, each curve for the Y-process obtained at three different retinal regions, rises with increasing intensities of light, the trend of curves being concave upward above the light threshold.

The rod-responses increase to some extent as the intensity of illumination rises, but decrease above a certain intensity. The critical intensity at which the decrease of rod-responses begins is lower for 5°, 20° and 50° in order, that is, the nearer the retinal location to the fovea, the more liable the rod-process to depression. This fact is another evidence that the depression of rod-activity is directly connected with cone-activity.

In the next series of experiments, the effect of retinal location was investigated more systematically. The results are shown in Fig. 4, in which the magnitudes of Y- and rod-processes are plotted against distances from the fovea. For comparison, the density distribution of rods determined by Østerberg[22] is represented by a dotted curve in each diagram. Measurements were performed at four different intensity levels of the pre-illuminating light.

The magnitude of the Y-process gradually decreases with increasing distance from the fovea at each brightness level.

As the curves obtained at 0.12 and 3 lux indicate, the magnitude of rod-response increases very steeply in the parafovea, reaches a maximum at about 10° to 20° from the fovea, and then gradually decreases toward

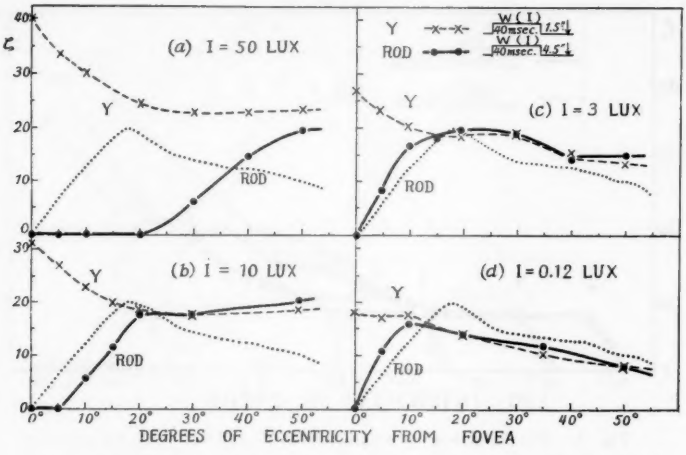


Fig. 4. Spatial distribution of Y- and rod-processes (temporal horizontal meridian of left retina). Full and broken curves represent magnitudes of Y- and rod-processes respectively. Dotted curve indicates density distribution curve of rods obtained by Østerberg. Intensity of light is given in each diagram.

the periphery. This distribution has an approximate resemblance to the density distribution of rods, though they are not identical with each other. When the intensity of light increases to 10 lux, the rod-responses take nearly constant values from the midperiphery to the far-periphery, showing no tendency to decrease toward the periphery like the density distribution of rods. The difference from the density distribution becomes more marked when the intensity is raised to 50 lux, as is shown in Fig. 4a. At this intensity the rod-activity is so completely depressed at the parafovea and the midperiphery of the retina that the spatial distribution curve of rod-response is completely different from the density distribution curve.

From all these findings it is very likely that the rod-activity is depressed in proportion to the number of cones activated.

Discussion

As to the function of rods in photopic vision, the following three possibilities have been propounded: A) The rods act as blue receptors in the manner that Roaf[4] and Willmer[5-10] assumed. B) The rods are either slightly active or inactive in photopic vision. C) They are as active as in scotopic vision.

If the theory by Roaf and by Willmer were correct, it would be dif-

ficult to understand the fact that in our experiment the rod-process and the blue process can be distinguished very clearly from each other. As was shown in a previous paper[14], the rod-process shows a spectral distribution well corresponding to the scotopic visibility curve and never shows a sensitivity maximum at the blue part of the spectrum under all experimental conditions. Therefore, the possibility A may be excluded. The possibility C may also be excluded, because our experiments indicate that the rod-process is completely suppressed above a certain intensity of light.

Yonemura and Ishizaka[23] obtained by the electrostimulation method similar results. Although their experiments were not systematic enough concerning retinal location, area and intensity of illumination etc., they found stronger inhibitory action upon the rod-process with increasing intensities and area illuminated.

Oikawa and Kurosawa[14] found in a previous paper that some notches appeared on the rod-response curves determined with spectral lights of high intensities and were ascribed to some inhibitory action of cones upon rods. The effective wavelengths to elicit cone-processes are different according to the kind of cone-processes. So it is quite probable that there are some wavelengths which are especially effective to inhibit the rod-activity. The notches found on the spectral response curve of rods is an expression of such selective inhibitory actions upon the rod-activity.

As is well known, the total color-blindness is always associated with photophobia. Since the cones must be slightly active or inactive in totally color-blind subjects, the inhibitory action of cones upon rods must be incomplete. Hence, the photophobia in these subjects may be due to unrestricted, exessive excitation of the scotopic mechanims. The inhibitory action of cones upon rods may be one of the natural defense mechanisms. But for this inhibitory mechanism, the extremely photosensitive receptors, rods would easily be exhausted.

As assumed by several authors,[16][24-25] retinal processes revealed by our method represent physiological events in the intermediate zone or retinal network. Polyak[26] provided histological evidence for lateral connections in the retina mediated by horizontal cells and by amacrine cells. The horizontal cells send their impulses in the centrifugal direction to receptor cells, cones and rods, while the amacrine cells send their impulses in the centripetal direction to a large number of ganglion cells. From these synaptic connection it is likely that the amacrine cells mediate spatial summation of retinal excitation, while the horizontal cells convey such inhibitory impulses from the cone-system to the rod-system as mentioned above. The inhibition through this channel seems to be most economical of energy, because excitation is suspended at the very entrance of the visual pathway, that is, at the receptor-cell level.

SUMMARY

By the method of electrostimulation, the cone- and rod-processes caused by white light were separately investigated at various parts of the retina.

1. As the intensity of light increased, both cone- and rod-processes increased in magnitude, but at a certain intensity the rod-process began to decrease, while the cone-process continued to rise.

2. The rod-process was completely suppressed above a certain intensity. The intensity for complete suppression was found different depending upon retinal location.

3. The rods nearer to the fovea were found to be more liable to suppression. In other words, the stronger the activity of cones, the greater the depression of rod-activity.

4. Since the rod-process could clearly be distinguished from the blue-process by their characteristic time constants, the theory that the rods would act as blue receptors in photopic vision, may safely be discarded.

5. It was discussed that the inactivity of rods in photopic vision may be due to an inhibitory action of cones upon rods.

We are greatly indebted to Prof. K. Motokawa for his kind guidance and for many valuable discussions.

References

1) Hecht, S., Physiol. Rev., 1937, **17**, 239.
2) Duke-Elder, W. S., Textbook of ophthalmology, Vol. 1, London, Henry Kimpton, 1932.
3) Lythgoe, R. J., Brit. J. Ophthal., 1940, **24**, 21.
4) Roaf, H. E., Physiol. Rev., 1933, **13**, 43.
5) Willmer, E. N., Nature, 1943, **151**, 213.
6) Willmer, E. N., *ibid.*, 1943, **151**, 632.
7) Willmer, E. N., *ibid.*, 1943, **152**, 190.
8) Willmer, E. N., *ibid.*, 1944, **153**, 774.
9) Willmer, E. **V.**, Brit. Med. J., 1950, **2**, 1141.
10) Willmer, E. N., J. Physiol., 1949, **111**, 179.
11) Brooke, R. T., J. Opt. Soc. Amer., 1951, **41**, 1010.
12) Oikawa, T., Tohoku J. Exp. Med., 1953, **57**, 109.
13) Parsons, J. H., An introduction to the study of colour vision, Cambridge Univ. Press London, 1924.
14) Oikawa, T. & Kurosawa, T., Tohoku J. Exp. Med., 1953, **59**, 333.
15) Wright, W. D., Researches on normal and defective colour vision, Henry Kimpton, London, 1946.
16) Motokawa, K., J. Neurophysiol., 1949, **12**, 291.
17) Motokawa, K., Tohoku J. Exp. Med., 1951, **54**, 385.
18) Motokawa, K. & Ebe, M., Tohoku J. Exp. Med., 1951, **54**, 215.

19) Motokawa, K., Ebe, M., Arakawa, Y. & Oikawa, T., J. Opt. Soc. Amer., 1951, **41**, 478.
20) Oikawa, T., Tohoku J. Exp. Med., 1953, **58**, 69.
21) Motokawa, K., J. Neurophysiol., 1949, **12**, 465.
22) Østerberg, G., Acta Opthal., 1935, **13**, Suppl. VI.
23) Yonemura, T. & Ishizaka, N., Rinsho Ganka (In Japanese), 1952, **6**, 154.
24) Talbot, S. A., J. Opt. Soc. Amer., 1951, **41**, 918.
25) Thomson, L. C., Ophthalmic Literature, 1952, **6**, 3.
26) Polyak, S., The retina, Chicago Univ. Press, Chicago, 1941.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Studies on Melanin

XXII. Nevus Fusco-caeruleus Acromio-deltoideus

By

Minor Itô
(伊藤 實)

From the Department of Dermatology, Tohoku University, Sendai; Director: Prof. M. Itô

(Received for publication, May 25, 1954)

The following case was described as the 18th case of nevus spilus en nappe[1] in my previous publication. Recently I had the opportunity to observe the biopsy of this patient. According to the quite coincidence of this histological findings to those of nevus fusco-caeruleus, the case here presented again is reported as a new type of nevus, namely nevus fusco-caeruleus acromio-deltoideus.

The patient, a girl, aged 17 noticed congenital pigmented macula on the area from left acromion to extensor surface of left upper arm. In the upper half of the macula diffused flecks thin blue in color were prominent and in the lower half portion its color tone decreased in grade and moreover many freckle-like spots were scattered over there. The pattern resembles one of a missprinted trichromatic illustration. The localization quite corresponded to the distribution of Nn. supraclaviculares posteriores and N. cutaneous brachii lateralis. No sensory disturbances were noticed. Slight gradial and chronometrical differences in local sweating than surroundings and the late development of erythema and wheal in histamine scratch test rather than normal skin were noticed. From these evidences local disturbances of vegetative nervous system were suggested. Histological findings quite same as Yoshida[2] reported in nevus fusco-caeruleus ophthalmo-maxillaris Ota.

To summarize, the transition in distribution of the blue and brown flecks *i.e.* meso-and ectodermal origin respectively suggested the topographical difference in the peripheral course of nerve fiber and may be a datum which suspects the participation of nervous system in the melanin formation.

References

1) Itô & Hamada, this journal, 1952, **55**-Supplement, 44 (Plate X, Fig. 41).
2) Yoshida, *ibid.*, 34 (Plate IV, Fig. 15, 16; Plate VIII, Fig. 31–33).

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Biochemical Studies on Carbohydrates

CLXIII. A Spectrophotometric Determination Method of Hexoses in Sugar Mixtures and Polysaccharides

By

Hajime Masamune and **Kiyosi Ogawa**
(正宗 一) (小川 清)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, August 2, 1953)

Hitherto have been applied carbazole,[1),2),3)] tryptophane[4)] or cysteine[5)] together with sulfuric acid to spectrophotometric analysis of hexoses. The first two agents give rise to absorption maxima at one and the same wave-length or at wave-lengths not far remote from one another for different hexoses, which does not allow accurate determination of individual sugars in mixture. The last agent is superior in this respect, because, in " SCyR I " of the author Dische, galactose causes very intensive absorption at 600 mμ as compared with other hexoses, although all hexoses including galactose show absorptions in the ultraviolet (between 320 and 400 mμ) with neighbouring maxima in " PCyR II."

We have found that thionalide induces with galactose an intensive absorption with maxima at 605 and 495 mμ by the aid of sulfuric acid, but with other hexoses glucose, fructose and mannose absorptions with a maximum at the latter wave-length alone. That absorption available for analysis occurs in the visible region for any hexoses and moreover that only one single reaction is requisite for the analysis of various hexoses must be expedient.

Experimental

Technique of the Determination Procedure and Explanation

Reagents. a) Thionalide pro analysi (Takeda). It is dissolved in 98% sulfuric acid immediately before use.

b) Sulfuric acid extra pure (Mitsubishi Kasei). It must not contain a contamination giving a green colour with an alcoholic α-naphthol solution.

Apparatus. Spekker-photometer (Shimazu).

Procedure. 0.5 cc. of a solution containing 0.05–0.175 mg. of the unknown in terms of total hexoses is pipetted into a test tube 19 mm.

diametral and 170 mm. long and immersed in ice water, and 3.0 cc. of 98% H_2SO_4 containing 1.0 mg. thionalide is added slowly along the wall. The mixture is shaken gently in the ice water for a few minutes and after standing for following few minutes, placed for exactly 3 minutes in actively boiling water. The mixture is thereupon quickly cooled in ice water and afterwards stood in such a big box as 90 cm. × 60 cm. × 60 cm. at 20°C. The absorptions at 605 and 495 mμ and, if necessary, that at 520 mμ are measured after 80 and 100 hours in a 1 cm. thick cell.

Explanation of the reaction course. The primary reaction of hexoses (galactose, mannose, glucose and fructose) give pale yellow products and that of pentoses (xylose, D- and L-arabinose and D-ribose) pale brown products, all with an absorption maximum in the ultraviolet. Within 30 minutes after heating, no absorption is seen at 495 and 605 mμ, provided that the total hexoses in the sample amount to no more than 0.2 mg. Methylpentoses (L-fucose and rhamnose) and acetylglucosamine give no color and accordingly the visible region is not absorbed, when 0.2 mg. or less as a whole of these sugars is submitted to the reaction, but brownish coloration arises by more than 0.2 mg. of them with an absorption maximum in the ultraviolet. Samples rich in lipid or protein particularly those colored in themselves often show absorption at 495 and 605 mμ, which remains constant after 30 minutes. These extinctions have to be determined at the 30th minute to subtract from the values obtainable after the completion of the secondary reaction (S. below). The primary reaction disappears gradually and at the same time the secondary reaction begins to appear, wherein the absorptions in the cases of mannose, glucose and fructose have a maximum at 495 mμ and that in the case of galactose maxima at 495 and 605 mμ. The color nuance of the solutions changes in parallel with the progress of reaction, and the reaction of free hexoses reaches completion in 66–78 hours and that of polysaccharides 96 hours after heating. Thereafter the constant absorption continues for about two days followed by gradual decrease. Fig. 1 shows the temporal courses of the absorptions at 495 or 605 mμ for hexoses. In Figs. 2, 3 and 4 are constructed the absorption curves of various sugars and sugar mixtures extending over a range of wave-lengths after full development of the secondary reaction, which takes also 66–78 hours after heating, indicating that the products of pentoses absorb light most intensively at 470 and 530 mμ and fairly at 495 mμ and those of acetylglucosamine and fucose scarcely at around 500 mμ and longer wave-lengths. Hence, if a pentose or pentoses are present in the unknown, their contents must be determined additionally to calculate hexose values (Regarding pentose determination, a procedure will be informed elsewhere[6]).

Calculation. Absorptions at 605 mμ by the products of the hexoses

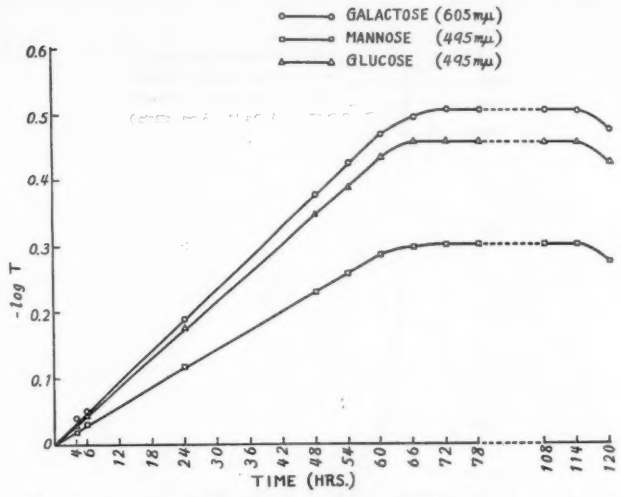


Fig. 1. Increase of absorption with time after heating with thionalide and sulfuric acid of 0.1 mg. of hexoses.

other than glactose and of other classes of sugars are neglectable so that the galactose amount is read immediately by the extinction at that wave-length from the curve in Fig. 5. The value enables one to read the extinction at 495 mμ for the same hexose, which is to be subtracted from the total extinction here for calculating the amount of mannose, glucose or fructose. If two of these sugars, for instance, glucose and mannose are present in addition to galactose in a sample, the individual values of glucose and mannose may be obtained as follows, although very strict analysis is *sine qua non*. At first the extinction corresponding to galactose at 495 mμ is subtracted from the total extinction here (E). The ratio of extinction at that wave-length for glucose E_{495}^{gl} to that for mannose E_{495}^{man} is 4.6/3.0=1.53 (Cf. Table I), namely, the sum of glucose and mannose expressed as mannose amounts to mannose plus $1.53 \times$ glucose. Be $\left(\frac{E_{495}}{E_{520}}\right)^{unk}$, $\left(\frac{E_{495}}{E_{520}}\right)^{man}$ and $\left(\frac{E_{495}}{E_{520}}\right)^{gl}$ the quotients for the unknown, mannose and glucose respectively, the molecular ratio of glucose to glucose plus mannose is given by dividing $\left[\left(\frac{E_{495}}{E_{520}}\right)^{unk} - \left(\frac{E_{495}}{E_{520}}\right)^{man}\right]$, which is denoted by x, with $\left[\left(\frac{E_{495}}{E_{520}}\right)^{gl} - \left(\frac{E_{495}}{E_{520}}\right)^{man}\right]$ which equals 0.19. Namely, $\frac{\text{glucose}}{\text{glucose}+\text{mannose}}$ equals $\frac{x}{0.19}$, and glucose $\frac{x \times \text{mannose}}{0.19-x}$. Since E_{495}^{gl} is

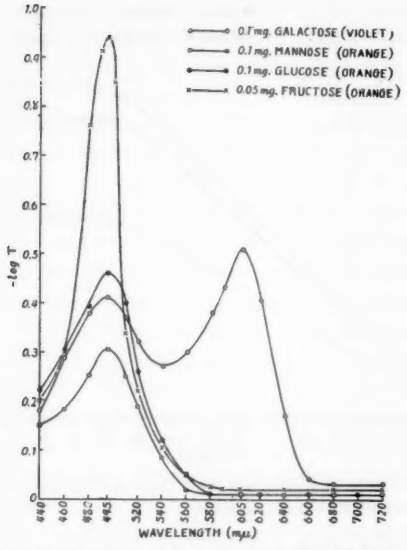


Fig. 2. Absorption spectra for hexoses constructed with the figures in Table I. The colours of the reaction mixtures at full development are noted in parentheses after the names of respective sugars in the explanation.

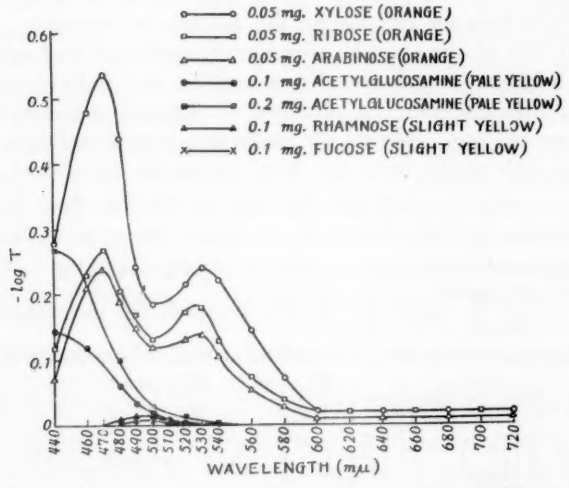

0.05 mg. XYLOSE (ORANGE)
0.05 mg. RIBOSE (ORANGE)
0.05 mg. ARABINOSE (ORANGE)
0.1 mg. ACETYLGLUCOSAMINE (PALE YELLOW)
0.2 mg. ACETYLGLUCOSAMINE (PALE YELLOW)
0.1 mg. RHAMNOSE (SLIGHT YELLOW)
0.1 mg. FUCOSE (SLIGHT YELLOW)
- log T
WAVELENGTH (mμ)

Fig. 3. Absorption spectra at full development (measuredtf a er 100 hours from heating) for various sugars other than hexoses. The colours of the reaction mixtures are described in the explanation as in Fig. 2.

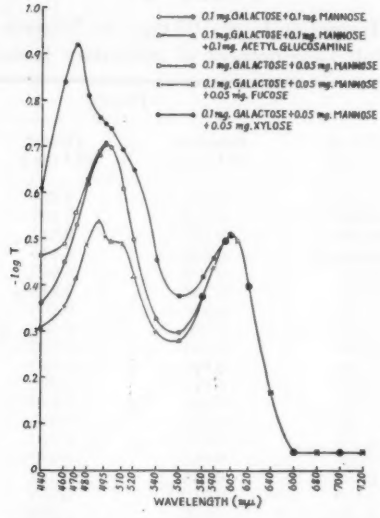

0.1 mg. GALACTOSE + 0.1 mg. MANNOSE
0.1 mg. GALACTOSE + 0.1 mg. MANNOSE + 0.1 mg. ACETYL GLUCOSAMINE
0.1 mg. GALACTOSE + 0.05 mg. MANNOSE
0.1 mg. GALACTOSE + 0.05 mg. MANNOSE + 0.05 mg. FUCOSE
0.1 mg. GALACTOSE + 0.05 mg. MANNOSE + 0.05 mg. XYLOSE
- log T
WAVELENGTH (mμ)

Fig. 4. Absorption spectra for sugar mixtures at full development (measured after 100 hours from heating).

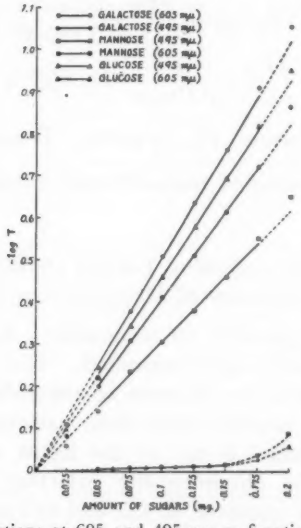


Fig. 5. Extinctions at 605 and 495 mμ as functions of the amount of galactose, mannose and glucose, measured at between 80th and 100th hour after heating the reaction mixtures.

TABLE I

Extinctions for Hexoses (0.1 or 0.05 mg.) at Various Wave-lengths after Full Development of Secondary Reaction

Wave-length mμ	Hexose			
	galactose (0.1 mg.)	mannose (0.1 mg.)	glucose (0.1 mg.)	fructose (0.05 mg.)
440	0.179	0.150	0.220	0.200
460	0.288	0.182	0.305	0.300
480	0.378	0.253	0.392	0.762
490	0.404	0.298	0.444	0.913
495	0.412	0.304	0.460	0.940
500	0.404	0.298	0.448	0.850
510	0.370	0.250	0.400	0.338
520	0.324	0.189	0.260	0.220
540	0.269	0.083	0.120	0.104
560	0.298	0.020	0.050	0.050
580	0.380	0.010	0.010	0.025
590	0.433	0.010	0.010	0.020
600	0.504	0.010	0.010	0.020
605	0.510	0.010	0.010	0.020
610	0.507	0.010	0.010	0.020
620	0.404	0.010	0.010	0.020
640	0.170	0.010	0.010	0.020
660	0.040	0.010	0.010	0.020
680	0.030	0.010	0.010	0.020
700	0.030	0.010	0.010	0.020
720	0.030	0.010	0.010	0.020

$\frac{4.6}{3}\times E^{man}_{495}$, $\left(E^{gl}_{495}+E^{man}_{495}\right)$ becomes $\left(\frac{4.6}{3}\times\frac{x}{0.19-x}+1\right)\times E^{man}_{495}$. Accordingly, if E is divided with $\left(\frac{4.6}{3}\times\frac{x}{0.19-x}+1\right)$, E^{man}_{495} is given, and if the latter is subtracted from the former, E^{gl}_{495} is given. From these partial extinctions are read the amounts of mannose and glucose from the curves in Fig. 5.

Experiments regarding Factors controlling the Accuracy of Determination

a) Effect of the quantity of thionalide. Amounts varying from 0.5 to 3.0 mg. of thionalide were examined. The absorptions of hexoses at 495 mμ increased with the increase of thionalide concentration, but simultaneously those of hexoses other than galactose at 605 mμ became manifest when more than 1.0 mg. of the agent was applied, entailing intricated calculation for obtaining the galactose value. Fig. 6 shows the extinction curves for mannose at 495 and 605 mμ and that at 605 mμ for galactose which is steep as compared with the corresponding curve for mannose. The durations of time till completion of the reaction of

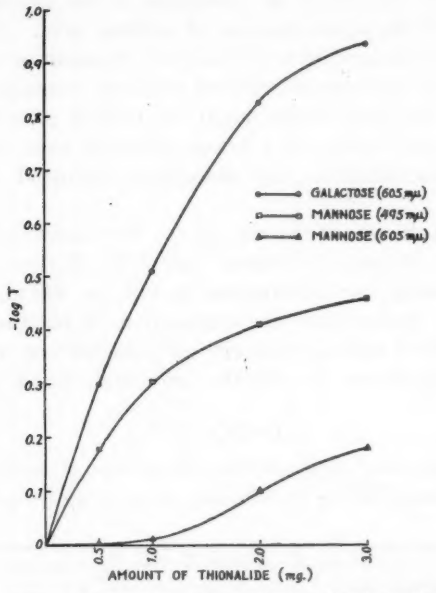


Fig. 6. Effect of thionalide concentration on the absorptions for 0.1 mg. of galactose and of mannose.

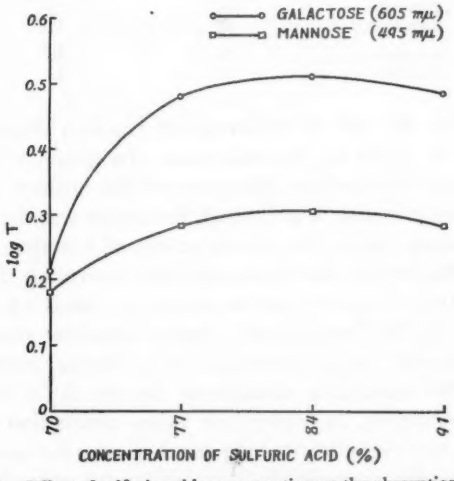


Fig. 7. Effect of sulfuric acid concentration on the absorptions for 0.1 mg. of galactose and of mannose.

free hexoses were not varied by alteration of the amount of the agent.

b) Effect of the concentration of sulfuric acid. The sulfuric acid concentration was changed from 70% to 91% by addition of 2.50 to 3.25 cc. of 98% H_2SO_4 to various volumes of solutions containing 0.1 mg. of a hexose so that the both fluids might be totaled 3.5 cc. When 3.0 cc. of 98% H_2SO_4 and 0.5 cc. of a hexose solution were mixed, that is, at 84% H_2SO_4 concentration, the absorption occurred most intensively (Fig. 7).

c) Effect of the size of the test tube. Test tubes of sizes (A) 16 mm. $\times$150 mm., (B) 19 mm.$\times$170 mm. and (C) 27 mm.$\times$170 mm. were examined, by taking the absorptions at 605 or 495 mμ as indications. In Table II are given hours for completion of reaction. In Tube A, the reaction with a definite quantity of galactose or mannose did not always reach completion in definite time and, besides, the coefficient

TABLE II

Time (hrs.) Requisite for Completion of Reaction

The absorptions at 605 mμ (for galactose) and at 495 mμ (for mannose) were measured

Test tube	Hexose taken	Amount of hexoses (mg.)		
		0.05	0.10	0.15
A	galactose	66–78	72–84	78–90
	mannose	66–78	72–84	78–90
B	galactose	66	72	78
	mannose	66	72	78
C	galactose	36	48	60
	mannose	36	48	60

of absorption after the full development of reaction changed by $\pm$0.05. Next in Tube B or Tube C, the maximum absorption took place in one and the same time, when equal quantities of the hexoses were examined, although the more a hexose was applied, the longer was the time necessary. In the latter of these tubes the development of reaction occurred more rapidly than in the former, but the maximum absorption did not continue long (not more than 6 hours) and its intensity varied ($\pm$0.06) from one run to another. In the former tube, nearly consistent maximum absorptions were shown for equal quantities of a hexose (variation $\pm$0.02). Further to add, the maximum absorptions did not differ according to the tube width, nevertheless, in wider test tubes absorption extended over a wider range of wave-lengths, making cumbersome the accurate measurement of galactose, for example, E_{605} values for 0.1 mg. of mannose were 0.005, 0.010 and 0.035 respectively in Tubes A, B and C.

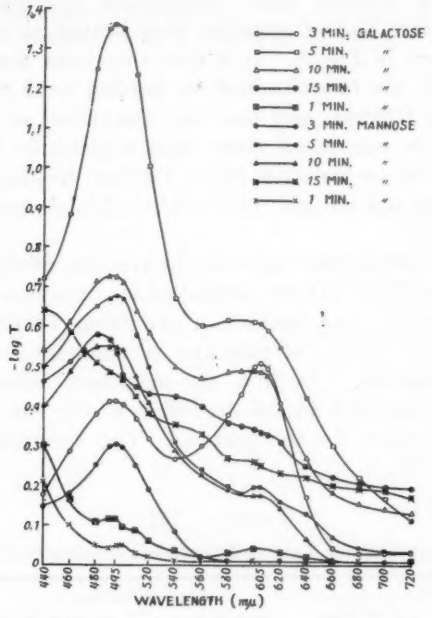


Fig. 8. Effect of heating time on the absorptions for 0.1 mg. of galactose and of mannose.

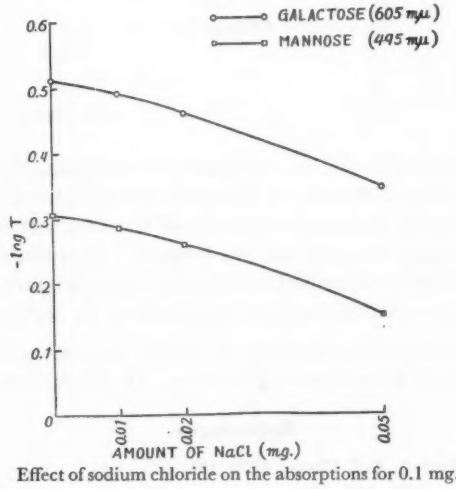


Fig. 9. Effect of sodium chloride on the absorptions for 0.1 mg. of galactose and of mannose.

d) Effect of heating time. Absorption spectra when reaction mixtures of galactose and of mannose were heated for various durations of time are plotted in Fig. 8. It is seen therefrom that, of the spectra for galactose, only the one obtained by heating for 3 minutes possessed a distinct peak at 605 mμ, and that the absorption at the same wavelength for mannose was much more than neglectable in cases of 5–15 minutes heating and, on the other hand, 1 minute heating of the mannose-containing mixture did not give rise to niticeable absorption even at 495 mμ.

e) Effect of temperature whereat the reaction mixture is maintained after heating. In Table III are embodied the durations of time for completion of the reaction and maximum extinction coefficients when the mixtures containing 0.1 mg. of galactose or mannose are stood in boxes at various temperatures. At 30°C the maximum absorption continued only for a short time and varied according to the run (±0.05), hence 20°C is more adequate for the purpose. The absorption at 10°C was weaker than at 20°C.

TABLE III

Reaction at Various Temperatures after heating the Mixtures

Hexose (0.1 mg.) taken	Temperature of the reaction mixtures (°C)	Max. extinction at		Time for full reaction (hrs.)
		605 mμ	495 mμ	
Galactose	10	0.410		102
	20	0.510		72
	30	0.608 (mean)		72
Mannose	10		0.245	96
	20		0.304	72
	30		0.355 (mean)	72

f) Effect of NaCl. 0.5 cc. of solutions containing 0.01, 0.02 or 0.05 mg. NaCl in addition to 0.1 mg. of a hexose were subjected to the analytical procedure, processing spectrophotometry of the reaction mixtures against the blank containing the salt but no hexose. As is illustrated in Fig. 9, the reaction was depressed by NaCl, for which chloride ions must have been responsible because of deficiency of such effect in sodium acetate.

Through the Grant Committee for Scientific Researches was given a grant from the Ministry of Education in aid to us. H. Masamune.

References

1) Gurin & Hood, J. Biol. Chem., 1939, **131**, 211.
2) Seibert, *ibid.*, 1946, **163**, 511.

3) Holzman, MacAllister & Niemann, *ibid.*, 1947, **171**, 27.
4) Shetlar, Foster & Everett, Proc. Soc. Exp. Biol. Med., 1948, **67**, 125.
5) Dische, J. Biol. Chem., 1950, **183**, 489.
6) Masamune & Ogawa, Tohoku J. Exp. Med., in press.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Chédiak and Higashi's Disease, Preliminary Report

Probable Identity of Chédiak's and Higashi's Diseases

By

Akira Sato*

(佐藤　彰)

From the Department, Pediatrics, Tohoku University, Sendai

(Received for publication, June 4, 1954)

Higashi published a congenital malformation of peroxidase granules in an infant in 1953, for which he suggested the name "Congenital Gigantism of Peroxidase Granules."[1] This is, I believe, the very first case of congenital peroxidase malformation. Then I came across an article[2] by Mois Chédiak, who described in 1952 a new familial and constitutional leucocytal anomaly, though he did not mention any peroxidase or oxidase reaction. It is highly probable that Chédiak's anomaly and Higashi's disease are at least two variations of one and the same disease.

	Higashi's case	Chédiak's case
A.	Etiology: Consanguinity. Only albinic sibs affected.	Same as in Higashi's case.
B.	Symptoms: Uneventful until general infection: acute leukemic syndrome with anemia, *leukopenia* and thrombopenia, further with hepatosplenomegaly.	Same as in Higashi's case.
C.	Prognosis: Succumbs invariably to the infection, so that mother can prophesy the inevitable death of the victim, who can never live beyond 3 years of age.	Same as in Higashi's case. Only the victim can never lived beyond 7 years of age.
D.	Treatment: Penicillin is of no avail.	Sulfonamides are of no avail.
E.	Pathognomonic blood sign :—	
	1. Peroxidase picture[3]: Nearly every granulocyte shows gigantic peroxidase granules (Fig. 1, b).	1. Peroxidase not examined.
	2. Giemsa stain: Many lymphocytes have each an inclusion body (Fig. 2, b). Myeloblasts show a large inclusion body.	2. Giemsa stain: Each lymphocyte has an inclusion body Myeloblasts as in Higashi's. case.

Fig. 1. Peroxidase (Neutrophiles)

a. Normal　b. Gigantic granules.

Fig. 2. Giemsa stain (Lymphocytes)

a. Normal　b. With inclusion body

"Chédiak and Higashi's Disease" or "Chédiak-Higashi's Leuco-anomaly" is suggested. The details will appear in a coming number of this Journal.

References

1) Higashi, Tohoku J. Exp. Med., 1954, **59**, 315.
2) Chédiak, Rev. Hemat., 1952, **7**, 362.
3) Sato & Sekiya, Tohoku J. Exp. Med., 1926, **7**, 11; Wintrobe, Clinical Hematology, Lea & Febiger, Philadelphia, 1952, p. 280.

* Professor emeritus.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Biochemical Studies on Carbohydrates

CLXIV. Spectrophotometric Determination of Pentoses in Sugar Mixtures and Polysaccharides

By

Hajime Masamune and **Kiyosi Ogawa**
(正宗　一)　　(小川　清)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, August 2, 1953)

Dische[1] employed cysteine to spectrophotometrically determine pentoses. Processed according to his technique, the density increment between 390 mμ and 425 mμ corresponds accurately to the amount of a pentose in a sample.

We recommended thionalide for analysis of hexoses.[2] This agent has been found to be of value for the analysis of pentoses as well, because it reacts with this class of sugars, showing absorption spectra in the visible region available for the purpose.

Technique and Principle of Analysis

Reagents. a) Thionalide pro analysi (Takeda).
b) Sulfuric acid extra pure (Mitsubishi Kasei) without a contamination giving a green colour by α-naphthol.

Apparatus. Spekker-photometer (Shimazu).

Procedure. 0.5 cc. of a sample solution containing 0.08 mg. or less of a pentose or pentoses as a whole is pipetted into a 19 mm. $\times$ 170 mm. test tube and immersed in ice water, then 3.0 cc. of a 86.5% sulfuric acid are added slowly along the wall. The mixture is shaken gently with continuous cooling in ice water, and after standing in air for following few minutes, heated in a vigorously boiling water-bath for exactly 3 minutes. It is cooled in ice water immediately thereupon, and when cold, 0.3 cc. of 86.5% sulfuric acid containing 1.0 mg. thionalide is added. The mixture here is placed vertically in an incubator of size 90 cm. $\times$ 60 cm. $\times$ 60 cm. at 20°C. After 48 hours, the extinctions at 540 and 580 mμ are measured in a Spekker-photometer.

Calculation. The value of a pentose is read from a curve of $E_{540}-E_{580}$ increments in Fig. 5 (plotted from Table I). If a pentose is present in a sample but no sugar else, the pentose is idenfied by $\frac{E_{540}}{E_{580}}$ which differs

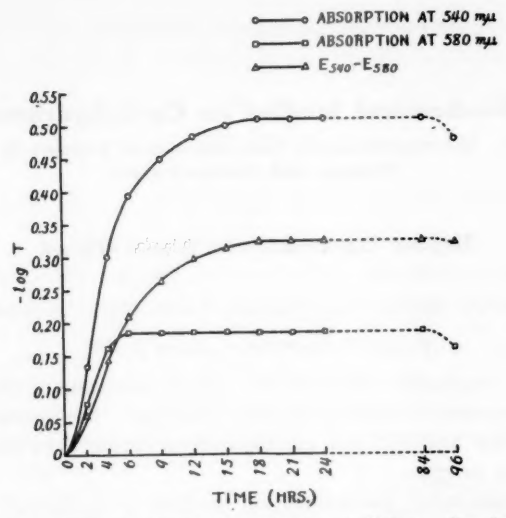


Fig. 1. Temporal change of extinction coefficients after heating of the reaction mixture containing 0.05 mg. of xylose.

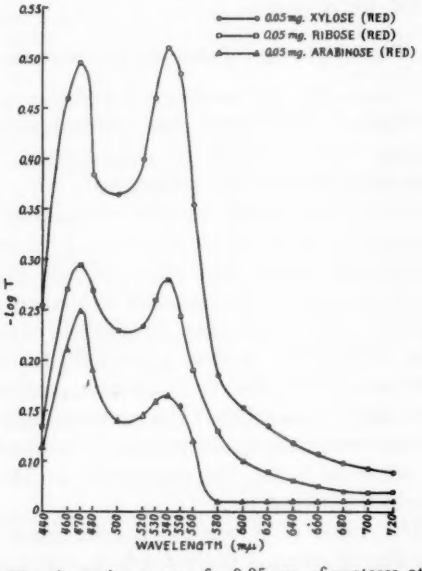


Fig. 2. The absorption spectra for 0.05 mg. of pentoses at full development. In parentheses after the names of the pentoses are given the colours of the reaction mixtures.

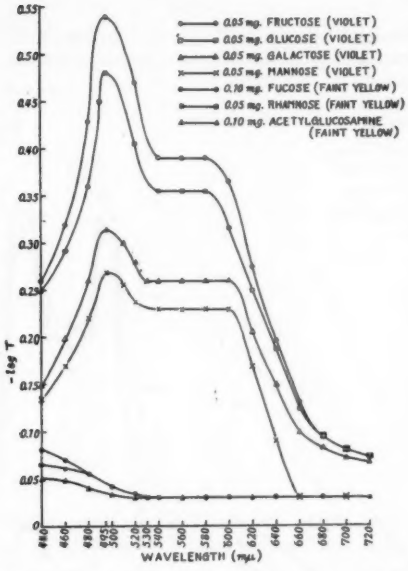

0.05 mg. FRUCTOSE (VIOLET)
0.05 mg. GLUCOSE (VIOLET)
0.05 mg. GALACTOSE (VIOLET)
0.05 mg. MANNOSE (VIOLET)
0.10 mg. FUCOSE (FAINT YELLOW)
0.05 mg. RHAMINOSE (FAINT YELLOW)
0.10 mg. ACETYLGLUCOSAMINE (FAINT YELLOW)
- log T
WAVELENGTH (mμ)

Fig. 3. The absorption spectra at full development for hexoses and sugars of other classes. The colours in parentheses after the names of sugars are those of the reaction mixtures.

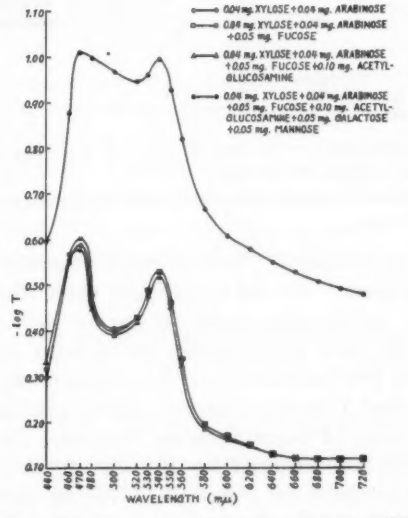

0.04 mg. XYLOSE + 0.04 mg. ARABINOSE
0.08 mg. XYLOSE + 0.04 mg. ARABINOSE + 0.05 mg. FUCOSE
0.04 mg. XYLOSE + 0.04 mg. ARABINOSE + 0.05 mg. FUCOSE + 0.10 mg. ACETYL-GLUCOSAMINE
0.04 mg. XYLOSE + 0.04 mg. ARABINOSE + 0.05 mg. FUCOSE + 0.10 mg. ACETYL-GLUCOSAMINE + 0.05 mg. GALACTOSE + 0.05 mg. MANNOSE
- log T
WAVELENGTH (mμ)

Fig. 4. The absorption spectra for sugar mixtures at full development.

Fig. 5

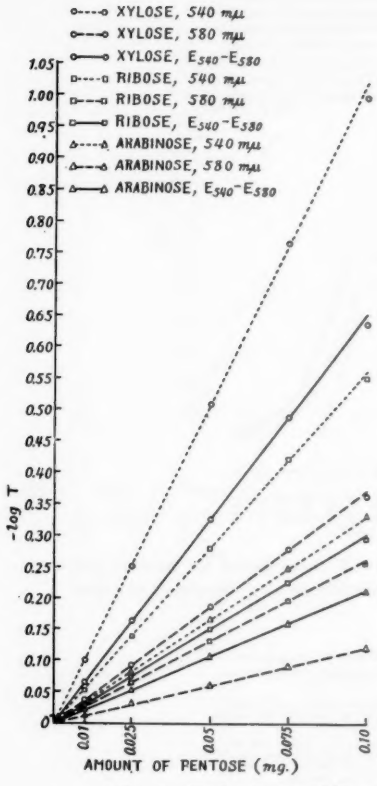


Fig. 6

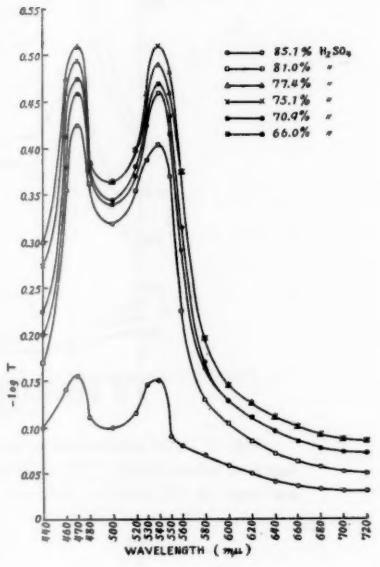


Fig. 5. Extinctions at 540 and 580 mμ and $E_{540}-E_{580}$ increments as functions of the amount of pentoses.

Fig. 6. Effect of sulfuric acid concentration on the absorption spectrum for 0.05 mg. of xylose.

from one pentose to another. Two or more pentoses in a sample are expressed as one of them. L- and D-arabinose show identical absorptions. Probably the same relationship holds in any of such pairs.

Explanation of the course of the reaction and principle of the analysis. The reaction mixtures of free pentoses get colored pale orange after 1 hour and turn red after another 1 hour. The red colour gradually deepens and reaches maximum after 18 hours from the heating. In the case of polysaccharides 48 hours are required for the full development. Then the absorption remains constant for 3 days, followed by steady disappearance (Fig. 1). Figs. 2–4 show the absorption spectra for pentoses, sugars be-

TABLE I

Extinctions for Pentoses at 540 and 580 mμ and $E_{540}-E_{580}$ Increments

Pentose Name	Pentose wt. (mg.)	Extinction at 540 mμ	Extinction at 580 mμ	$E_{540}-E_{580}$
Xylose	0.01	0.100	0.035	0.065
	0.025	0.250	0.090	0.160
	0.05	0.510	0.185	0.325
	0.075	0.765	0.280	0.485
	0.10	0.995	0.360	0.635
Ribose	0.01	0.055	0.025	0.030
	0.025	0.140	0.065	0.075
	0.05	0.280	0.130	0.150
	0.075	0.420	0.195	0.225
	0.10	0.550	0.255	0.295
Arabinose	0.01	0.035	0.015	0.020
	0.025	0.085	0.033	0.052
	0.05	0.165	0.060	0.105
	0.075	0.257	0.100	0.157
	0.10	0.330	0.120	0.210

longing to other classes and their mixtures after completion of reaction (which took 18 hrs. in all cases). The curves for pentoses show two absorption maxima at 470 and 540 mμ, and those for hexoses one maximum at 495 mμ and a step in a range from 540 to 580 mμ or from 530 to 600 mμ. Methylpentoses and acetylglucosamine give rise only to slight absorption at the wave-lengths 530 mμ and less, and the absorption at more than 530 mμ is negligible. The spectra in Fig. 4, which have been experimentally obtained, prove to be combinations of those for the individual sugars in mixture, although the co-presence of acetylglucosamine appears to somewhat depress the absorption due to pentoses, and reveal moreover that the difference between the extinctions at 540 and 580 mμ, where the extinctions for a hexose are equal with one another, always agrees with that for the pentose or pentoses constituting a sugar mixture or a polysaccharide. As is seen from Fig. 5, pentose amounts 0.08 mg. and less have a linear relationship with the density increments corresponding to them.

Factors controlling the Reaction

a) Effect of the concentration of sulfuric acid. 98.0% H_2SO_4 was diluted with $\frac{1}{20}$, $\frac{1}{10}$, $\frac{1}{7.5}$, $\frac{1}{5}$ and $\frac{1}{3.5}$ the volume of water. And the reaction mixtures were prepared with 3 cc. of 98.0% H_2SO_4 as such or one of the diluted, otherwise similar to above, so as to contain 85.1 (I), 81.0 (II), 77.4 (III), 75.1 (IV), 70.9 (V) and 66.0% (VI) sulfuric acid re-

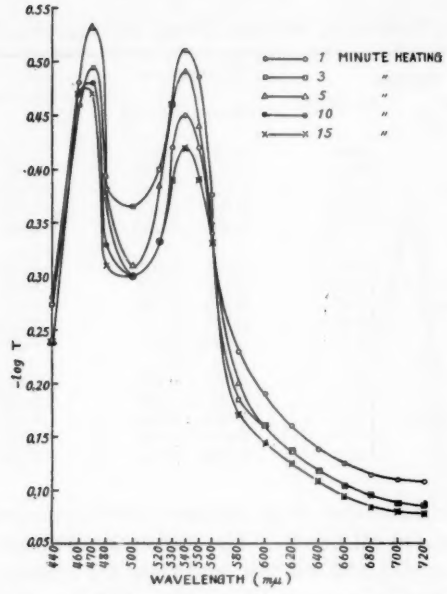


Fig. 7. Effect of heating time upon the absorption spectrum for 0.05 mg. of xylose.

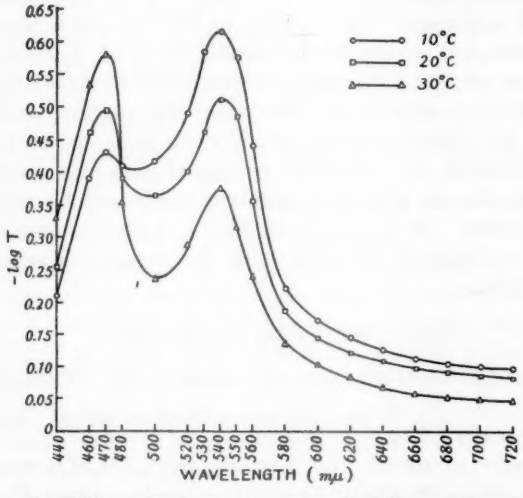


Fig. 8. Effect on the absorption spectrum for 0.05 mg. xylose of chamber temperature to which the reaction mixture is exposed after heating.

spectively and 0.05 mg. of xylose. The results are given in Fig. 6 and Table II. It is seen that, in the case of IV, where sulfuric acid used was of the same concentration as in the reaction mixture described in the item " Procedure," the absorption at 540 mμ and the increment between 540 and 580 mμ showed largest values.

TABLE II

Reactions when Sulfuric Acid Concentration was varied

0.05 mg. xylose was examined.

	No. of reaction mixtures					
	I	II	III	IV	V	VI
$E_{540}-E_{580}$	0.055	0.275	0.305	0.325	0.300	0.295
Time for completion of reaction (hrs.)	42	36	24	18	18	18
Colour at full development of reaction	faint orange	red	red	red	red	red

b) Effect of heating time. Fig. 7 shows the absorption spectra for 0.05 mg. xylose when time of heating was varied from 1 to 15 minutes. In any of the cases, the reaction was brought to completion in 18 hours developing red colour. The $E_{540}-E_{580}$ values amounted to 0.220, 0.325, 0.290, 0.250 and 0.250 respectively when heating was continued for 1, 3, 5, 10 and 15 minutes. Namely 3 minutes' heating is most suited to the measurement because of the largest $E_{540}-E_{580}$ with the most intensive absorption at 540 mμ.

c) Effect of chamber temperature to which the reaction mixture is exposed after heating (Fig. 8). At 10°, 20° and 30°C, 18 hours were always the time till full development of the reaction (with red coloration), and the increments between 540 and 580 mμ were respectively 0.395, 0.325 and 0.255 which all diverged according to the run by ±0.01 only. 10° must be most fitted for the analysis, provided that the chamber can be fixed at that temperature accurately throughout the year.

d) Effect of the thionalide concentration. By increase of the amount of thionalide above 1.2 mg., $E_{540}-E_{580}$ was suddenly augmented. Judging from the curve showing the increments (Fig. 9), slightly inaccurate weighing of the agent does not cause any significant analytical error within the limits 1.2 and 0.5 mg. of it.

e) Effect of the size of the test-tube. Three test-tubes of sizes 16 mm. × 150 mm. (A), 19 mm. × 170 mm. (B) and 27 mm. × 170 mm. (C) were examined. The time till the completion of reaction varied (A: 24 hrs., B: 18 hrs., C: 18 hrs.), but duration of following constant ab-

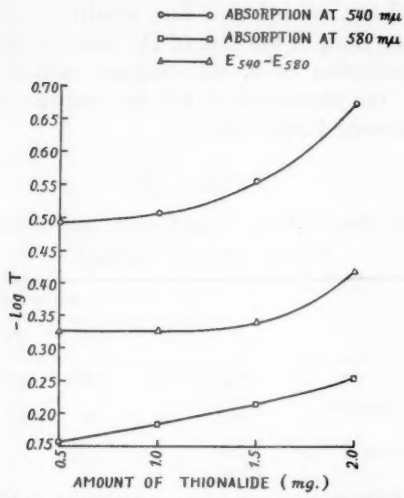


Fig. 9. Effect of thionalide concentration on the absorptions at 540 and 580 mμ for 0.05 mg. of xylose.

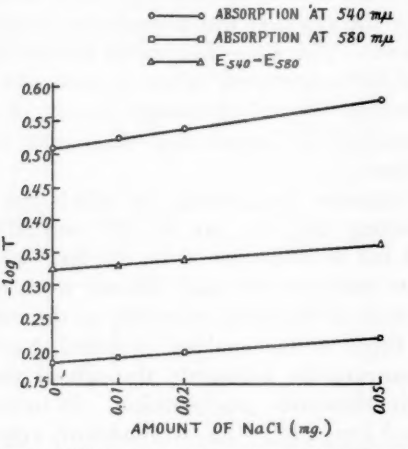


Fig. 10. Effect of NaCl on the absorptions at 540 and 580 mμ for 0.05 mg. of xylose.

sorption and the increment $E_{540}-E_{580}$ did not.

f) Effect of chloride ions. Addition of NaCl induced some increase of E_{540}, E_{580} and $E_{540}-E_{580}$ as is shown in Fig. 10, whereas sodium acetate was of no influence. (In the blank with a water sample the same quantity of sodium chloride or acetate was added as in the main.)

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References

1) Dische, J. Biol. Chem., 1949, **181**, 379.
2) Masamune & Ogawa, Tohoku J. Exp. Med., this volume, p. 11.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Biochemical Studies on Carbohydrates

CLXV. A Spectrophotometric Determination Method of Metylpentoses in Sugar Mixtures and Polysaccharides

By

Hajime Masamune and **Kiyosi Ogawa**
(正宗　一)　　(小川　清)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, August 2, 1953)

To the end, Nicolet & Shinn[1], titrated acetaldehyde produced by periodic acid oxidation of the sugars. Toricelli[2] colorimetrically determined furfural and total aldehyde in the distillate after hydrolysis by H_3PO_4 of a sample to calculate out the amount of the sugar from the difference of the two aldehyde values. But those methods require not only a comparatively large amount of the material but also fair skill. Later on, Dische & Shettles[3] introduced cysteine-H_2SO_4 reaction of this class of sugars and spectrophotometrically measured the absorption in the ultraviolet. We have found that thionalide is more expedient than cysteine for the spectrophotometric analysis because the reaction product shows absorption in the visible region.

Technique

Reagents. Thionalide pro analysi (Takeda) in 50% H_2SO_4, which in its turn is prepared with H_2SO_4 extra pure (Mitsubishi Kasei) free from a contamination giving green colour with α-naphthol.

35% HCl extra pure (Mitsubishi Kasei).

Apparatus. Spekker-photometer (Shimazu).

Procedure. 0.5 cc. of a solution containing 0.1–0.4 mg. of a sample in terms of the methylpentose in it is pipetted into a test tube and immersed in ice water, and 0.3 cc. of 50% H_2SO_4 containing 1.0 mg. of thionalide (This reagent must be freshly prepared) and 3 cc. of 35% HCl added in sequence with continuous shaking and cooling. When cold, the mixture is removed from the ice water, and after standing in air for a few minutes, heated in a vigorously boiling water-bath for accurately 5 minutes, followed by cooling in ice water. It was then placed in a large incubator (90 cm. $\times$60 cm. $\times$60 cm.) at 20°C. The reaction of free methylpentoses reaches full development in 3 hours and that of methylpentoses in polysaccharides in 4–5 hours, and the maximum absorption continues for at least 6 hours

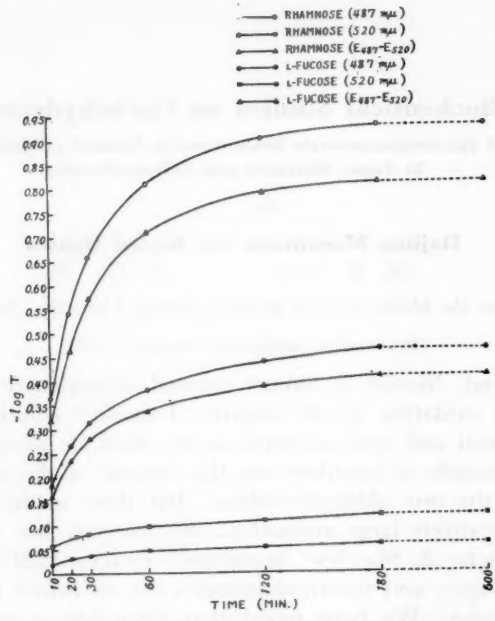


Fig. 1. Change of absorption intensity with time, when 0.3 mg. portions of rhamnose and of L-fucose were submitted to the reaction.

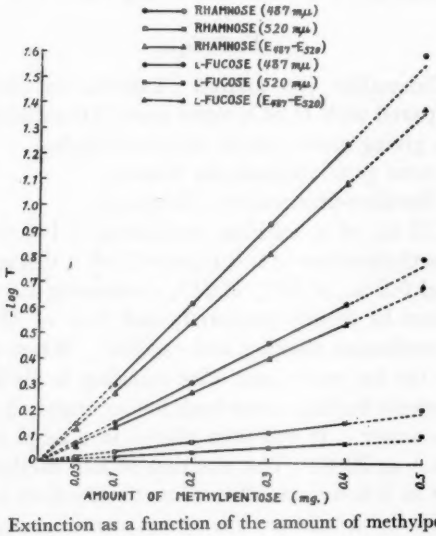


Fig. 2. Extinction as a function of the amount of methylpentose.

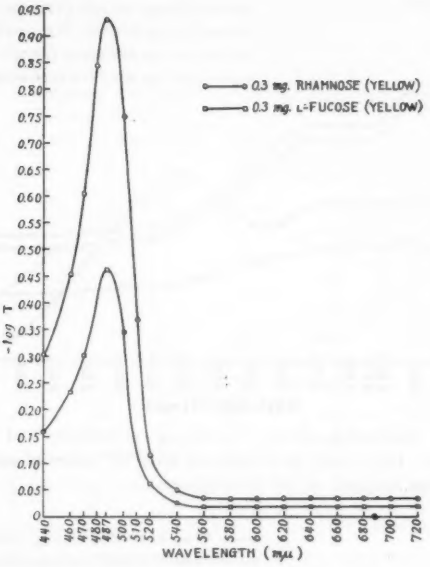


Fig. 3. Absorption spectra for 0.3 mg. of methylpentoses. The colors in parentheses after the names of sugars in the explanation are those of the reaction mixtures at full development.

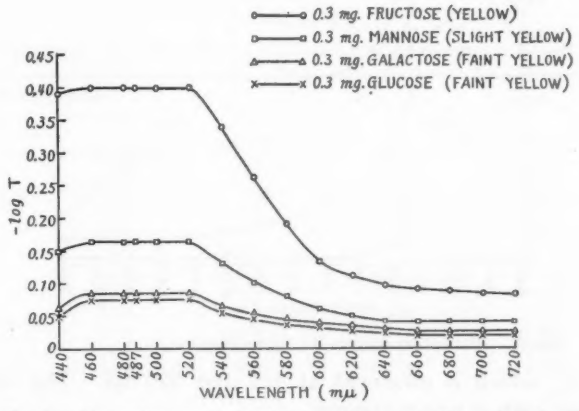


Fig. 4. Absorption spectra for 0.3 mg. of hexoses. The colors in parentheses after the names of sugars are those of the reaction mixtures at full development.

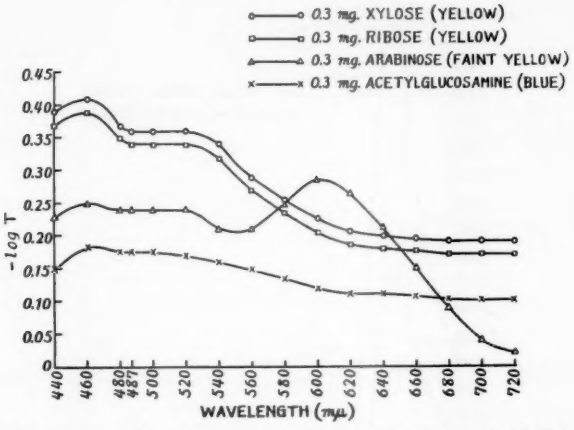


Fig. 5. Absorption spectra for 0.3 mg. of pentoses and of N-acetylglucosamine. The colours in parentheses after the names of sugars are those of the reaction mixtures at full development.

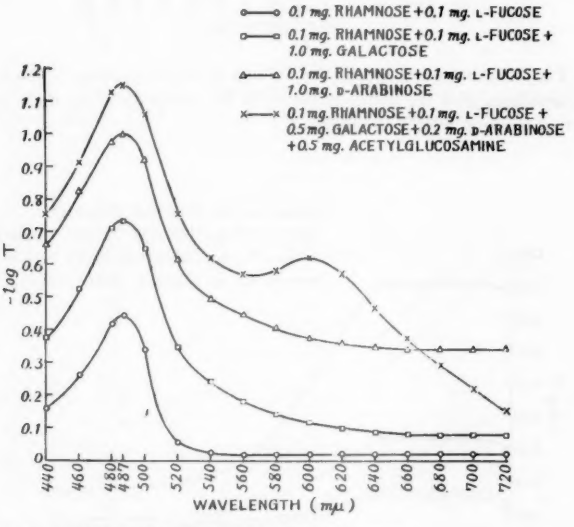

0.1 mg. RHAMNOSE+0.1 mg. L-FUCOSE
0.1 mg. RHAMNOSE+0.1 mg. L-FUCOSE+ 1.0 mg. GALACTOSE
0.1 mg. RHAMNOSE+0.1 mg. L-FUCOSE+ 1.0 mg. D-ARABINOSE
0.1 mg. RHAMNOSE+0.1 mg. L-FUCOSE+ 0.5 mg. GALACTOSE+0.2 mg. D-ARABINOSE +0.5 mg. ACETYLGLUCOSAMINE
-log T
WAVELENGTH (mμ)

Fig. 6. Absorption spectra for sugar mixtures.

(S. Fig. 1), which is measured at 487 and 520 mμ. The blank run is carried out with a water sample.

Calculation. The increment $E_{487}-E_{520}$ is calculated and the sugar amount is read from one of the curves in Fig. 2 constructed from the

Fig. 7

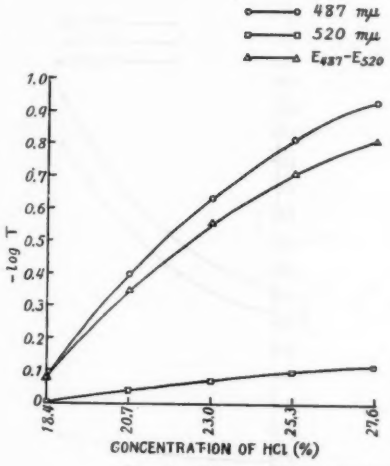


Fig. 8

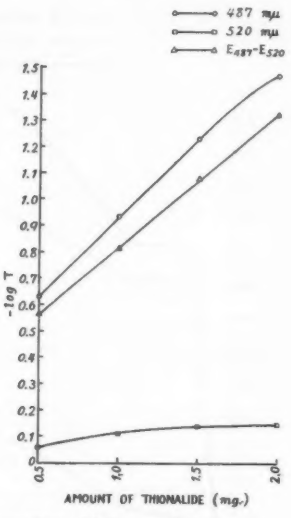


Fig. 7. Effect of HCl concentration on the reaction of 0.3 mg. of rhamnose.

Fig. 8. Effect of the thionalide amount upon the reaction of 0.3 mg. of rhamnose.

figures in Table I. Two or more methylpentoses are expressed as one of them.

TABLE I

E_{487}, E_{520} and Increment $E_{487}-E_{520}$ of Methylpentoses

Methylpentose name	wt. (mg.)	E_{487}	E_{520}	$E_{487}-E_{520}$
L-Fucose	0.05	0.066	0.010	0.056
	0.1	0.150	0.020	0.130
	0.2	0.312	0.040	0.272
	0.3	0.465	0.060	0.405
	0.4	0.622	0.078	0.544
	0.5	0.800	0.108	0.692
Rhamnose	0.05	0.145	0.020	0.125
	0.1	0.300	0.034	0.266
	0.2	0.622	0.079	0.543
	0.3	0.930	0.117	0.813
	0.4	1.245	0.155	1.090
	0.5	1.593	0.213	1.380

If a methylpentose is present as a sole sugar, its identification can be made from the ratio $\frac{E_{425}}{E_{520}}$, which differs from one methylpentose to another.

Fig. 9

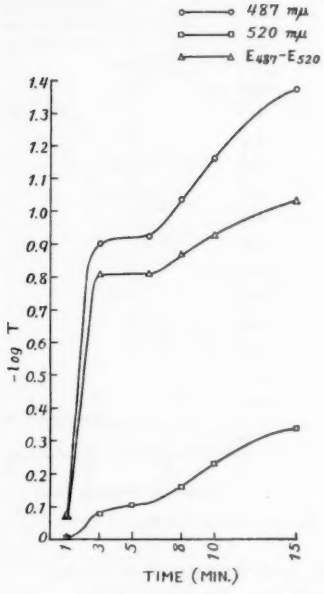


Fig. 10

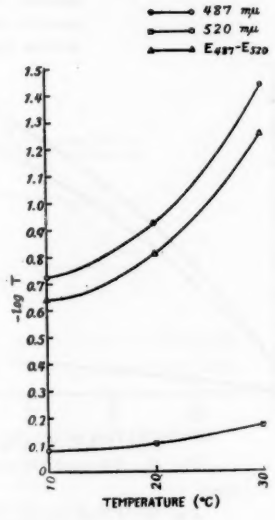


Fig. 9. Effect of heating time upon the reaction of 0.3 mg. of rhamnose.
Fig. 10. Effect of chamber temperature, whereto the reaction mixture is exposed after heating, on the reaction of 0.3 mg. of rhamnose.

For two methylpentoses without co-presence of any other kind of sugar, calculation of the amounts of the individual sugars is made by a manner resembling that for hexoses.[4]

Explanation of the Analysis and Remarks

Figs. 3–5 show that E_{487} is equal to E_{520} in hexoses, pentoses and acetylglucosamine contrary to methylpentoses, and Fig. 6 that mixtures of sugars of those classes with methylpentoses give by the reaction an absorption corresponding to the total sum of the absorptions for the individual sugars, $E_{487}-E_{520}$ always representing the sum of the increments for the methylpentoses only.

Following remarks are particularly to be noted. 1) Within the limits of 0.1 and 0.4 mg. of a methylpentose or the total methylpentose, the extinction follows the law of Lambert & Beer. 2) By adopting $E_{487}-E_{520}$ for calculation, the influences of lipids and proteins on the analysis can be ignored. 4) The extent of absorption as well as the reaction velocity is not affected by the size of the test-tube employed.

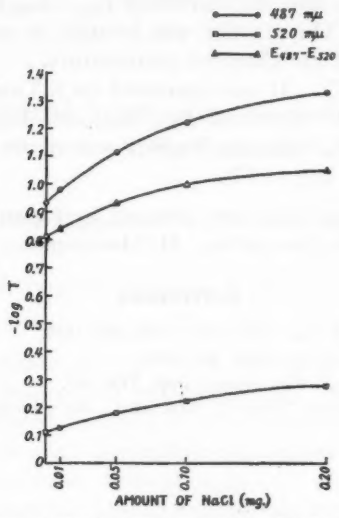


Fig. 11. Effect of NaCl upon the reaction of 0.3 mg. of rhamnose.

Experiments regarding Various Factors controlling the Reaction

1) Effect of hydrochloric acid concentration. The hydrochloric acid concentration in the reaction mixture was varied, without alteration of the total volume and else. As Fig. 7 shows, the lower the hydrochloric acid concentration was, the less were the absorptions at 487 and 520 mμ and their difference.

2) Effect of the concentration of thionalide. Fig. 8 illustrates the result, when various amounts (from 0.5 to 2.0 mg.) of thionalide were used together with 0.3 mg. of rhamnose. The absorptions E_{487} and E_{520} as well as the increment between those wave-lengths increased with the increase of thionalide in the reaction mixture.

3) Effect of heating time. Experiments with 0.3 mg. of rhamnose varying the heating time from 1 to 15 minutes gave the curves in Fig. 9. They show that $E_{487}-E_{520}$ increments nearly equal each other between 3 and 5 minutes of heating. Heating for more than 5 minutes gave rise to humin production and accordingly made difficult exact measurement of absorptions. The time till completion of reaction after heating was always 3 hours.

4) Effect of chamber temperature, whereto the reaction mixture is exposed after heating, upon the absorptions. Strict maintenance of the mixture at a definite temperature is requisite for the analysis, other-

wise not only E_{487} but also the increment $E_{487}-E_{520}$ changes extremely as is shown in Fig. 10. The reaction was brought to completion always in 3 hours irrespective of the chamber temperature.

5) Effect of NaCl. It was examined on 0.3 mg. of rhamnose (Fig. 11). Addition of NaCl increased E_{487}, E_{520} and $E_{487}-E_{520}$ in harmony with the finding in 1), whereas Na_2SO_4 was of no influence. The effect of NaCl must be due to Cl^-.

The expenses of this work were defrayed by a grant from the Education Department for Scientific Researches. H. Masamune.

References

1) Nicolet & Shinn, J. Am. Chem. Soc., 1941, **63**, 1456.
2) Toricelli, cited in C. A., 1946, **40**, 3370.
3) Dische & Shettles, J. Biol. Chem., 1948, **175**, 595.
4) Masamune & Ogawa, Tohoku J. Exp. Med., this volume, p. 11.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Biochemical Studies on Carbohydrates

CLXVI. On Pentose Determination in Nucleic Acids

By

Hajime Masamune and **Kiyosi Ogawa**
(正宗　一)　　(小川　潔)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, August 2, 1953)

In a foregoing paper we reported a thionalide method[1] for quantitative estimation of pentoses in polysaccharides. In continuation of the study we have attempted to apply it to pentosenucleic acids.

As is already known, pyrimidine-nucleosides and -nucleotides are hydrolyzed incompletely by mineral acids due to the firm N-glycoside linkage, so that immediate application of the procedure to them can not give satisfactory pentose values. Gurin and Hood[2] found by the carbazole reaction that bromination makes both uridine and uridylic acid more readily hydrolyzable by the "sulfuric acid-reagent," but the ribose values of uridylate and yeast nucleic acid thus obtained were still far remote from theory, notwithstanding that in uridine the value amounted beyond 90% of the calculated (They brominated 3–4 mg. of nucleic acids with 2–3 drops of the sat. bromine water in an ice bath for 5 minutes). Later on, Massart and Hoste[3] subjected a mixture of adenosine and cytidine to bromination (at 105°C for 2 minutes) and hydrolysis (The solution freed from bromine was stood at 105°C for 30 minutes) prior to the orcinol analysis according to Barrensheen and Peham.[4] The extinctions measured corresponded to 93–99% of the calculated. We investigated the conditions for preparatory bromination of pentosenucleosides to determine pentose in them with the thionalide method, and further attempted to hydrolyze nucleic acids with a dilute ammonia before bromination, because pentose in nucleic acids is not estimated so quantitatively as that in nucleosides by the procedure for the latter.

Experimental

Requisite Conditions for Adequate Bromination

Crystalline guanosine, adenosine and cytidine were prepared according to Levene and Jacobs[5][6] and amorphous uridine according to Steudel and Peiser,[7] starting with yeast nucleic acid of E. Merck.

24 to 28 mg. of those nucleosides and sodium nucleate from yeast (E. Merck) were dissolved in 16 cc. of distilled water or in the same volume of 0.1 *N* H_2SO_4 (in the case of gnanosine or the nucleate). From each of the solutions six 2 cc. portions were separately pipetted into 10 cc. volumetric flasks, and adjusted to pH 4.0 with 1 drop of 0.1 *N* H_2SO_4, excepting those of guanosine and nucleate solutions, then two of the flasks were placed in ice water, two immersed in a boiling water-bath and one in a thermostat at 20°C, and after addition of a slight excess of the saturated bromine as below, shaken gently for 5 or 10 minutes with continuous exposing to the respective temperatures. For those solutions kept in ice water, that in the thermostat, that in boiling water for 5 minutes and that in boiling water for 10 minutes were necessary 1 (0.03 cc.) (1 drop of the half-sat. bromine water was insufficient, because bromine was soon decolorized), 2, 3 and 4 drops respectively of the saturated bromine water to give persistent coloration. Thereafter, the solutions were chilled in ice water and excessive bromine expelled off by aeration through a capillary tube for 15 to 30 minutes. The capillaries were rinsed into the respective flasks, and after filling up to the mark with water or with 0.1 *N* H_2SO_4 (in the case of guanosine), 0.5 cc. portions of the solutions here were taken for the pentose determination by means of thionalide.

The results are embodied in Table I, which shows that marked increase of the pentose value occurred in uridine, cytidine and the nucleic

TABLE I

Determination of Ribose in Yeast Nucleic Acid and Nucleosides from it with and without Previous Bromination

In parentheses are given percentages of the theoretical.

Substance	Ribose content in per cent						
	theoretical	analyzed without previous bromination	analyzed after bromination				
			in ice water		at 20°C for 5 min.	in boiling water	
			for 5 min.	for 10 min.		for 5 min.	for 10 min.
Guanosine*	47.0	45.7	44.8 (95.3)	42.1	39.2	37.0	28.8
Adenosine†	50.8	49.5	48.6 (95.7)	46.6	42.8	39.9	30.3
Cytidine	61.5	47.6 (77.4)	55.4 (90.1)	51.3	43.0	40.4	31.7
Uridine	61.5	48.8 (79.3)	56.9 (92.5)	52.1	44.2	41.0	32.0
Yeast nucleic acid (sodium salt)‡	38.0§	25.0 (65.8)	32.1 (84.4)	30.5	23.4	21.0	15.6
D-Ribose	100.0	100.0	97.8	94.4	87.6	83.1	63.9

* Contains 2 molecules of crystal water. † Contains 1.5 molecules of crystal water.
‡ E. Merck. Dische diphenylamine reaction negative. § Calculated from P content.

acid, when bromination had been processed in ice water and particularly for 5 minutes, and that the value decreased in all the nucleosides and nucleic acid after bromine treatment at higher temperatures. Under the former conditions the prior treatment did not cause free ribose any significant change of the analytical value, whereas the higher the temperature and the longer the time were, the lower was the value obtained. It is thus most probable that excess bromine oxidizes the pentose, which has been set free at acid reaction from the unstabilized N-glycosides, into a product non-reactive with thionalide. Therefore, the smallest possible quantity of bromine must be employed at low temperature.

All of purines, pyrimidines and desoxypentosenucleic acids did not develop any colour in the thionalide reaction even after bromination. On direct submission to this color reaction, the yeast nucleic acid and all the pentosenucleosides showed a red color after 48 and 24 hours respectively, but after preliminary bromination under the various conditions above, they got colored within 18 hours like free pentoses.

Preliminary Hydrolysis with Dilute Ammonia[8]

The table above indicates that a pentose value more divergent from the theoretical is obtained in the nucleic acid than in the nucleosides, suggesting the necessity of preliminary hydrolysis of the acid for bromination. The conditions below were preferred after trials.

6 to 7 mg. of the yeast nucleate (sodium salt) are weighed into a tube and 2 cc. of 0.045% ammonia added to pH$>$9.8. The tube is sealed and heated in an autoclave for 3.5 hours at 130–140°C. Cooled. Guanosine crystals precipitate from the solution, which is uncolored and shows pH 8.6–8.8. The mixture is transferred to a 4 cc. volumetric flask, followed by filling up to the mark rinsing the tube with 0.1 *N* H_2SO_4 into the flask (guanosine crystals are dissolved by the sulfuric acid). 2 cc. of the solution here are brominated in ice water for 5 minutes as recommended above, and pentose determined.

Three nucleic acid preparations from different sources were thus analyzed. In all cases a red color of the mixture of the worked up unknown and H_2SO_4 gradually deepened in the course of 18 hours after addition of thionalide, and maximum absorption continued for about 48 hours. The analytical figures given are listed in Table II. From the result it became clear that pentose in nucleic acids is nearly quantitatively (93 to 96% of the value calculated from the phosphorus content) estimated.

An experiment as to the temperature for hydrolysis of nucleic acids will be appended. The yeast nucleic acid was heated at various tempera-

TABLE II

Analysis of Pentose in Nucleic Acids without Preliminary Hydrolysis and Bromination or with one or two of the Preliminary Treatments

Temperature of heating 130-140°C. In parentheses are given the percentages of the theoretical.

Substance	Pentose as ribose							P¶ %
		determined by the thionalide method						
	theoretical§ %	immediately		with prel. bromin.		with prel. hydrolys. and bromin.		
		%	pentose// / P	%	pentose// / P	%	pentose// / P	
Yeast nucleic acid (sodium salt)*	38.0	25.0	3.2	32.1	4.1	35.6 (93.7)	4.6	7.8
Hog kidney pentosenucleic acid (sodium salt)†	30.0	21.8	3.5	26.5	4.3	28.9 (96.3)	4.7	6.2
Hog stomach mucosa pentosenucleic acid‡	36.3	26.5	3.6	30.0	4.1	33.8 (93.1)	4.5	7.5

* The same sample as in Table I. † Prepared by Masamune *et al.*[9] ‡ Tokutomi's preparation.[10] § Calculated from P content. // Theoretically 4.8 ($=\frac{150}{31}$). ¶ Analyzed by a modification of Plimmer method.[11]

TABLE III

Effect of Hydrolysis Temperature on Ribose Analysis in Yeast Nucleic Acid

6.6 mg. of sodium nucleate of yeast were heated in the ammoniacal solution for 3.5 hrs.

Temperature °C	Ribose in per cent	Ribose / P
125–130	33.8	4.3
130–135	35.6	4.6
135–140	35.5	4.6
140–145	31.3	4.0
145–150	28.5	3.7

tures as is shown in Table III, with subsequent bromination and pentose analysis. The hydrolysis at about 135°C gives the most rational value.

SUMMARY

Conditions for hydrolysis and bromination of pentosenucleic acids

were investigated to apply the thionalide method for pentose determination to this class of compounds.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department, which enabled us to carry out this investigation. H. Masamune.

References

1) Masamune & Ogawa, Tohoku J. Exp. Med., this volume, p. 23.
2) Gurin & Hood, J. Biol. Chem., 1941, **139**, 775.
3) Massart & Hoste, Biochim. et Biophys. Acta, 1947, **1**, 83.
4) Barrensheen & Peham, Z. physiol. Chem., 1942, **272**, 81.
5) Levene & Jacobs, Ber. deut. chem. Ges., 1910, **43**, 3150.
6) Levene & Jacobs, J. Biol. Chem., 1916, **25**, 103.
7) Steudel & Peiser, Klein Handbuch d. Pflanzenanalyse, III, p. 458, Cited in Egami, Kakusan to Kakutanpakusitsu (Jap.), Kyoritsu Publishing Co., Tokyo, 1951, Vol. I, p. 113.
8) The procedure of Levene & Jacobs[5)6)] was somewhat modified.
9) Masamune, Tsuiki, Tokutomi & Kikuchi, Tohoku J. Exp. Med., 1954, **59**, 193.
10) Tokutomi, *ibid.*, 1953, **59**, 163.
11) Haneda, Sinokawa & Yasuoka, *ibid.*, 1944, **46**, 263.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Electrical Responses of the Human Brain to Acoustic Stimulus

By

Mituyosi Abe
(阿部 實賢)

From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai

(Received for publication, August 24, 1953)

Introduction

In 1939 P. A. Davis[1] reported that a transient potential change appeared in electroencephalogram of conscious human subjects upon beginning and cessation of a continuous intense sound. According to her observation, such an evoked potential was triphasic, having a latency of about 70 msec. and its amplitude was found maximal at the region of the vertex. Later a series of observation was made by H. Davis and his associates[2] in which the said evoked potential was found to increase in amplitude and duration as the subject became drowsy. Such a disturbance pattern evoked by a sound during the sleep has been called K-complex.

Since then, the evoked potential as found by P. A. Davis has not been an object of extensive studies. In conscious subjects, the electrical activity of the brain usually changes from moment to moment in a random manner. Under this condition the electrical response to a peripheral sensory stimulus may be almost completely masked or distorted by the fluctation of the background activity. Consequently sensory evoked responses in conscious human subjects are too obscure to be recorded clearly and no extensive study of their properties can successfully be carried out.

Recently a new technique to record evoked potentials from conscious subjects has been developed by Dawson.[3] He has used a recording system in which a sufficient number of records taken under the same experimental condition are superimposed in a single photograph. If a certain deflection follows a sensory stimulus with a regular latency, it is emphasized in a superimposed record against the randomly occurring background waves. Thus, the sensitivity of recording is appreciably increased and the accuracy of measurement is secured by a statistical treatment.

In the present experiment an attempt has been made to study evoked responses of the human brain to auditory stimulation making use of the Dawson's technique mentioned above.

Experimental

Method

Throughout the present experiment, brain potentials were picked up by a monopolar lead with a diffuse electrode on the earlobe. The electrodes were flattened pellets of soldar, about 3 mm. in diameter, and they were fixed to the skin with an electrode-paste and a courtplaster.

The amplifier used in the majority of experiment was of R-C-type, its frequency characteristics being almost flat in the range from 5 to 40 c.p.s. The recording instrument was an electromagnetic oscillograph.

As a source of continuous sound, a R-C-oscillator was used, the output voltage of which was applied to a vibrometer through a calibrated attenuater. When a click was to be used as a stimulus instead of a continuous sound, it was produced by passing a break-induction current from an inductorium through the vibrometer. A stethoscope transmitted the sound from the vibrometer to the ears of the subjects. A sound-proof room was not used in the present experiment.

Dawson's technique was conducted in the following way: all the oscillographic records obtained under the same condition of stimulation were graphycally superimposed upon the other, where the mark of stimulation in each record was carefully adjusted to a common mark.

Results

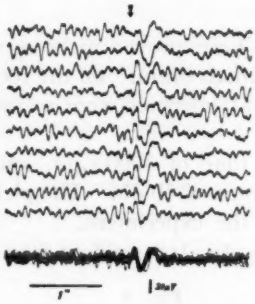
1. Wave forms of electrical responses to sound stimuli

In the first place we have confirmed Davis' finding that the evoked response to auditory stimuli could easily be discerned in the subjects whose EEGs were scant of alpha rhythms. Therefore the majority of the present experiment has been made with non-alpha type of subject. It was also observed that, even in these subjects, evoked potentials obtained under one and the same experimental condition could be different from one another in potential pattern. Such a situation may be seen from Fig. 1. In this figure a series of 10 records obtained with the same intensity of stimulus are arranged in such a manner that the starts of the stimuli in all records coincide with one another. From these records it seems that the evoked potential is triphasic, beginning with a sharp upward deflection. However, the exact form and magnitude of the response can not be known from these single records, because each record must have suffered from a deformation due to spontaneous activity. By superimposing all these records after the Dawson's technique the bottom tracing in Fig. 1 was obtained. As we can see a very clear triphasic response in this superimposed curve, it is now possible to describe the wave form of the evoked potential and to measure its latency, amplitude, etc.

In the case of alpha type of subjects it was often observed that the

effect of the auditory stimulus was a mere momentary distortion of the prevailing alpha rhythms as exemplified by the top record in Fig. 2. However, as will be seen from the middle tracing in this figure, the superimposition of 10 such records made the evoked potential clearly visible.

Fig. 1



Eig. 2

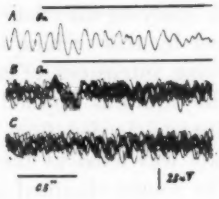


Fig. 1. A series of 10 records obtained from the vertex under the same condition of stimulation. The stimuli, 1000 c.p.s. at 100 db, were started at arrow. In all records triphasic potential changes are evoked soon after the onset of the stimulus, but they are different in pattern from each other due to background activities. By the method of superimposition the bottom tracing is obtained in which the evoked potential in question is seen clearly.

Fig. 2. Records from an alpha type of subject. A : on-effect of the sound appears as a mere distortion of the prevailing alpha waves. B : superimposed record with evoked potential visible more clearly. C : control for B without stimulus.

From the superimposed tracings in Fig. 1 and 2, it can be said that there is almost no qualitative difference in wave form of the evoked potential between the alpha and non-alpha type of subject. In general, the evoked potential to auditory stimulation is a triphasic potential change in which the scalp electrode becomes at first electrically negative, then positive and finally negative against the indifferent electrode on the earlobe. The duration of the excursion is shortest in the first deflection and longest in the last one. Concerning the amplitude, the second positive deflection was most prominent in all cases.

According to the intensity of stimulus and the region to be tested the above-mentioned shape of the evoked potential is modified in the manner as will be mentioned later.

As to the off-effect of the sound stimulus, Davis reported that the off-response did not occur so often as the on-response. I found, however, that the sound stimulus which was intense enough to give rise to the on-response could also bring about the off-response. An example of the

off-response is presented at the top of Fig. 3. In this figure it is also shown that the method of superimposition is useful to make the response in question visible. Since the off-response was found similar in character to the on-response, data concerning the on-response alone were presented in this paper.

2. Amplitudes and latencies of the evoked potentials in relation to the intensity of stimulus

As stated above, Dawson's technique has been proved to be serviceable for studying the evoked potential of the human brain. Of the records obtained by this technique the amplitudes and latencies were measured at varying intensities of the stimulus.

The stimulus applied was a continuous tone of 1000 c.p.s. and its intensity was varied from 40 to 100 db in steps of 10 db. The pick-up electrode was fixed at the vertex throughout the experiment.

The results obtained are illustrated in Fig. 4. It is evident from this

Fig. 3

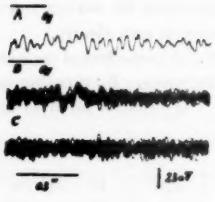


Fig. 4

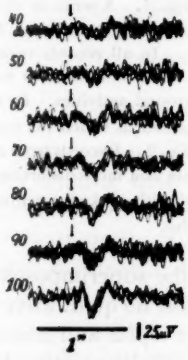


Fig. 3. Off-effect of the sound stimulus. A: an example of single record. B & C: superimposed records obtained with and without stimulus respectively.

Fig. 4. Series of records showing changes in pattern of evoked potentials caused by stimuli of varying intensities. Continuous tone of 1000 c.p.s. was used. The onset of the stimulus is marked by arrows. Numerals on left represent stimulus strength in db units.

figure that the amplitude of the evoked potential decreased gradually as the intensity of stimulus was reduced. With respect to the latency, it was slightly shortened as the intensity of stimulus increased. At the highest intensity used of the stimulus the onset of the first deflection of the evoked potential was found at about 90 msec. and the peak of the second positive deflection was attained at about 0.15 sec. after the onset

of the stimulus.

In Fig. 5 the amplitudes and the latencies of the positive deflection are plotted against the intensity of stimulus in db units. As can be seen from the diagram the relation between the amplitude and the intensity of stimulus can be represented by a straight line which, if extrapolated, passes through the zero point of the intensity, i.e. the threshold of audibility.

3. Distribution of the evoked potential over the scalp

It has been reported by Davis that the evoked response to the auditory stimulus was seen most clearly in the vertex than in any other regions. In order to make such a situation clearer we explored the surface of the scalp systematically and mapped out the distribution of the evoked response.

The stimulus used was a click of short duration and its intensity was fixed at a definite level. As areas to be tested we selected 16 points on one side of scalp as marked by dotts in Fig. 6.

Fig. 5

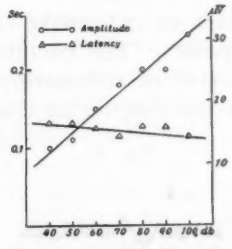


Fig. 6

Fig. 5. Amplitudes and latencies of positive components of evoked potentials are plotted as a function of stimulus intensity.

Fig. 6. Distribution of evoked potentials over the scalp. Note that the response is not localized in a particular region but diffusely distributed. Byoken line S.C. means the projection of central sulcus.

As the recording instrument available to us had only three channels, it was very cumbersome to obtain a sufficient number of records for applying Dawson's technique with respect to all the areas to be tested. Therefore we recorded the evoked response three or four times from one and the same region and only the typical record obtained was reproduced in Fig. 6. From this figure it is evident that the evoked potential is not localized in a particular region but diffusely distributed over the scalp.

The amplitude of the response was found maximal at the vertex and decreased with increasing distance from the vertex. It is to be noted that the response from the forehead is reverse in phase to those from the remaining regions. Such a feature can be clearly demonstrated by the method of superimposition as is illustrated in Fig. 7. In this figure two

tracings were presented, of which one was obtained from the glabella and the other from the temporal region. It is probable that the reversal of the phase at the forehead region is caused by the unresponsiveness of the frontal region to the auditory stimulus on the one hand and by the physical spread of the potential change to the earlobe on the other.

4. Refractoriness of the brain set up by the auditory stimulus

Up to the present, the evoked potential technique has been successfully been used for mapping the sensory receiving cortex in animals. It is to be noted that the evoked potential in these cases is quite different in properties from our present evoked response to auditory stimulation. In the first place, the former is localized in the particular sensory area, while the latter is widely distributed over the scalp as has been mentioned above. With respect to the latency, the difference between these two kinds of evoked potentials is quite obvious; the evoked potential which serves for mapping the sensory cortex of animals has a latency less than 10 msec. in almost all cases,[4-6] whereas the latency of the evoked potential in our experiment measures 90 msec. or more.

The following experiment was undertaken so as to make clearer the difference between these two kinds of responses. The essentials of the experiment consisted in estimating the period of refractoriness of the brain set up by the auditory stimulus. For this purpose the conditioning

Fig. 7

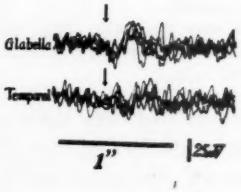


Fig. 8

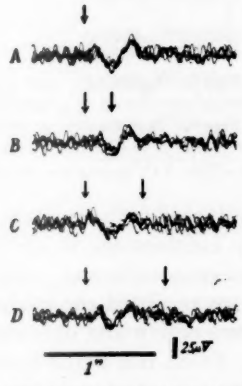


Fig. 7. The method of superimposition can clearly demonstrate that the response from the glabella is reverse in phase to that from the temporal region.

Fig. 8. A series of records illustrating the recovery of cortical excitability from the refractoriness following a strong single click. A: control record obtained by a single click. The time intervals between the conditioning and the testing click were 0.25, 0.5 and 0.75 seconds in B, C and D respectively. The response to the testing click can be found only in D.

and testing stimuli were delivered in succession at varying intervals. The stimuli were clicks of equal strength which were strong enough to give rise to maximal responses at the vertex. The results of this experiment are shown in Fig. 8. When the test stimulus was delivered less than 0.5 sec. after the conditioning one, there was no visible response to the test stimulus (tracing B and C in Fig. 8). When the interval between two stimuli was 0.75 sec., the response, though reduced in size, appeared upon the test stimulus (tracing D in Fig. 8). From this experiment it is evident that during the period of 0.5 sec. following the conditioning stimulus the brain was absolutely refractory to the test stimulus. It can also be said that the absolute refractoriness set up by the conditioning stimulus ended at least within 0.75 sec.

The same experiment as ours has been made by Jarcho[5] and by Chang[6] with anesthetized animals. In these experiments the evoked potentials localized in the sensory cortex have been taken as an index of the cortical excitability. According to their observations, the duration of the absolute refractoriness was not longer than 50 msec. in the deepst anesthesia and sometimes it was less than 10 msec. under the most favorable experimental condition. It is obvious that the period of refractoriness is much shorter in animal experiments by Jarcho and by Chang than in our experiment on human subjects.

5. Effect of anesthesia upon the evoked response

Davis and his associates have conducted an experiment in which the effect of the sound stimulus upon the electrical activity of the human brain was studied in the progress of the natural sleep. They found that the on-response to the sound gradually increased in amplitude and duration as the sleep became deeper. At the stage of sleep at which 14-per-sec. waves appeared in EEG, the on-response was greatest in size and longest in duration.

In our experiment in which the subject was anesthetized with pentobarbital sodium, it could be demonstrated that, as the anesthesia deepened, the evoked response to the sound stimulus changed in the manner similar to that in the natural sleep. An example of records is presented in Fig. 9. In this case the stimulus applied was a click generated in the way described above and the pick-up electrode was placed in the vertex. The response obtained in the conscious state of the subject is exemplified by the record A in Fig. 9. The response was so well-developed that it could easily be distiguished from the background activity. By administrating the solution of pentobarbital sodium to the subject, the stage of anesthesia was reached in which 14-per-sec. activity prevailed in EEG. Under this condition the sound stimulus could cause an evoked potential which had a greater amplitude and a longer duration than in the consciou

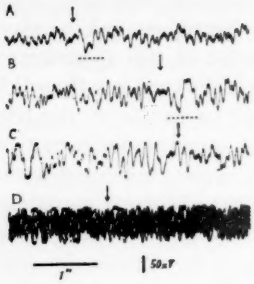


Fig. 9. Changes in potential pattern of evoked potential in progress of barbiturate anesthesia. A: normal control. B: stage of 14-per-sec. waves. The evoked potential was augmented and its duration lengthened. In A and B the evoked potentials are marked by the dotted lines. C: stage of slow waves. D: the method superimposition was applied to the records in the stage of slow waves. No visible deflection can be found. In all records clicks were applied at arrows.

state (record B in Fig. 9). By adding a small quantity of anesthetic the anesthesia was further pushed to the stage at which slow waves of large amplitude appeared in group between the train of 14-per-sec. waves. As will be seen from the record C in Fig. 9, no evoked response seemed to be elicited by the sound stimulus. Though sometimes an occasional deflection in form of the usual evoked potential appeared following the sound stimulus, the existence of the potential change having the definite wave form could not be proved by the method of superimposition (bottom tracing in Fig. 9).

Thus, it has been established that in the natural sleep as well as in the barbiturate anesthesia the evoked response to the sound stimulus increases in amplitude and duration and that it is most markedly augmented in the stage of 14-per-sec. waves. As has been confirmed by a number of investigators, the functional state of the brain in the barbiturate anesthesia is closely similar to that during the natural sleep. Therefore, it is conceivable that the modification of the evoked response takes place in the barbiturate anesthesia in the same manner as in the natural sleep.

When the sensory cortex of the animal is mapped by the evoked potential technique, it is an usual method of experimentation that the animal is maintained in a deeply anesthetized state. Though the spontaneous electrical activity is strongly suppressed by the anestheitc, the sensory receiving mechanism of the cortex is not affected. In contrast to this fact, the evoked response in our experiment is abolished in the stage in which the spontaneous waves are not yet completely suppressed.

6. Experiment of conditioning the evoked potential

In the majority of our subjects the sound stimulus could elicit no remarkable sensory response of the brain except the on- and off-responses which have been fully described in the above section. In some subjects, however, it was often observed that there appeared β-waves following the on-response upon the continuous sound. In such cases, it can be said that the sensory response of the brain consisted of on-response and

the following β-activity. These two sorts of electrical disturbances are similar to each other in that they are widespread over the scalp. However, the following experiment clearly shows that the nervous mechanism underlying these two kinds of sensory effects are quite different from each other.

The experiment consisted in conditioning the electrical activity of the brain with an auditory and non-auditory stimulus as an unconditioned and conditioned stimulus respectively. Result of this experiment is shown in Fig. 10. In this case an electric light of low intensity lasting for 11 seconds was used as a conditioned stimulus. The record 10 A was obtained

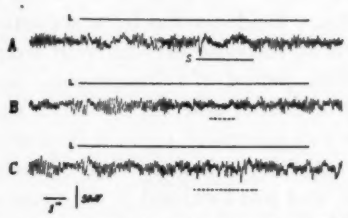


Fig. 10. Electroencephalographic conditioning of electrical responses of the brain to auditory stimulation. A : a conditioned stimulus (electric light of low intensity ; L) was followed by an unconditioned stimulus (strong sound of short duration ; S) at a time interval of about 5 seconds. Note that the sensory effect of the unconditioned sound stimulus consists of an evoked potential and a short train of β-waves. B : after a number of reinforcements, the effect of L alone was examined. Only β-activity appears as a conditioned response of the brain as indicated by the broken line. C : in the stage of the highest excitability of the brain the conditioned EEG response was θ-instead of β-waves. Even in this state no conditioned response in form of the usual evoked potential appears.

in a reinforcement in which an intense sound of 2.5 seconds in duration was applied at about 5th second from the start of the conditioned stimulus. As will be seen from this record, the unconditioned effect of the sound stimulus was such that an on-response of large amplitude was followed by a train of β-waves. The reinforcement was repeated at a regular time interval and somtimes the effect of the conditioned stimulus alone was examined. After a number of reinforcement we have found that the conditioned excitatory response of the brain appeared in a manner as is shown by the record 10 B. In this record a train of β-waves was elicited at a point of reinforcement in spite of the absence of the unconditioned stimulus. However it can scarcely be found that such train of β-waves was preceded by a potential change in form of the usual on-response. In some cases it was observed that a conditioned electrical activity was θ-instead of β-waves as will be seen from the record 10 C. According to the electroencephalographic study of conditioned reflexes by Motokawa[7] and by Iwama,[8] it is in the state of the highest excitability of the brain that a train of θ-waves appears as a conditioned activity of the human brain. In our cases, it was difficult to ascertain that even in the state of the highest excitation the conditioned stimulus alone could bring about a

potential change having a form of the usual on-response to the sound stimulus.

On the basis of these findings the following conclusion may be drawn. Though the on-response as well as the associated β-waves may be taken as the sensory effect of the sound stimulus upon the human brain, they are different from each other in that β-waves can be conditioned while the on-response can not.

Discussion

In the present experiment the properties of the evoked response of the human brain has been studied in comparison with those of the evoked response localized in animal's sensory cortex. Evidence has been accumulated which indicates that these two sorts of response are different from each other in latency, potential distribution, period of refractoriness, etc. In this respect, the experimental finding of Forbes and his associates deserves our attention. They could obtain two successive cortical responses following stimulation of the cat's sciatic with single shock. One of them could be elicited with a latency of 10 msec. and was localized in the sensory receiving cortex of the side contralateral to stimulation. Such a type of response has been called "primary response." It is evident that the cortical response picked up for mapping the animal's sensory cortex belongs to Forbes' primary response. The other sort of response, called "secondary discharge," is a delayed cortical response with a latency of 40 to 80 msec. In contrast to the primary response, it can be recorded from all the parts of the cortex.

It has been supposed by Davis that the evoked response of the human brain to the sound is identical with the secondary discharge in Forbes' term, because both phenomena were similar in property; two responses were not localized in a particular area of the brain and the latency was considerable in each case. Our data presented above also support Davis' supposition: The period of refractoriness estimated in our experiment is about 0.5 seconds or so, the value of the same order as has been found by Forbes[9] with respect to the secondary discharge of the cat's brain. In addition, it has been reported by Forbes *et al.* that their secondary discharge increases in amplitude as the anesthesia deepens. A similar feature has been found in our experiment on human subjects.

Now it seems very likely that our present evoked response of the human brain is analogous to Forbes' secondary discharge. Such being the case, it can be expected that the electrical response corresponding to Forbes' primary discharge must be detected in the human brain. The experiments were successfully carried out by Dawson and by Bates.[10] By the method of superimposition these authors could demonstrate that

a sensory discharge having a short latency of 20 msec. or so could be led off from the surface of the scalp near the central sulcus contralateral to peripheral sensory stimulation. It is without doubt that such a minute potential change as has been found by the above-mentioned authors belongs to the primary sensory evoked potential in human subjects.

Thus it has been established that the human brain can respond to sensory stimulation in the same manner as has been observed by Forbes on animals. In addition to this, the sensory stimulus can also give rise to β-acitivity in human subjects. Therefore, it can be said that there are at least three kinds of potential pattern in the sensory response of the human brain, i.e. primary response, secondary discharge and β-activity. However, β-activity must be distiguished from the former two potential patterns. While the primary and the secondary response are passive activities evoked only when the sensory stimulus is applied, β-activity is not merely a passive excitation of the brain, but also can represent an active cortical process. Such a circumstance can clearly be shown in our experiment of electroencephalographic conditioning: When the conditioning has advanced, conditioned β-activity is elicited by the conditioned stimulus alone as a result of an active cortical excitation.

Summary

The electrical response of the human brain to auditory stimulation was studied by means of Dawson's method of superimposition. The stimulus applied was a continuous tone of 1000 c.p.s. of short duration or a click of high intensity. Though the sound stimulus had on- as well as off-effect, the present experiment was concerned mainly with the on-effects.

1. The evoked response was triphasic activity beginning with the electronegativity of the pick-up electrode against the indifferent lead on the earlobe. At the highest intensity applied of the stimulus the first negative deflection was started at about 90 msec. after the onset of the stimulus and the peak of the second positive deflection was reached at about 150 msec. These values were not greatly reduced by increasing the intensity of stimulus.

2. The amplitude increased almost linearly with the stimulus intensity in db units.

3. The evoked potential was found widely distributed over the scalp, the amplitude being maximal at the vertex. The unresponsiveness of the forehead region was inferred from the finding that the response from that region was reverse in phase to those from the remaining regions.

4. The refractoriness of the brain following the sound stimulus was measured by applying two clicks of equal intensity as a conditioning and

testing stimulus respectively. It was found that the absolutely refractory period was about 0.5 seconds.

5. When a subject was anesthetized with a barbiturate, the evoked potential increased in size until the stage of 14-per-sec. activity was reached. At the stage of slow waves the evoked potential was lost sight of.

6. An experiment of electroencephalographic conditioning was made on a subject who responded to the sound stimulus with an evoked on-response followed by a short train of β-waves. A photic stimulus of low intensity and a strong sound of short duration were used as conditioned and unconditioned stimulus respectively. The time interval between two stimuli was about 5 seconds. After a number of application of the combined stimulus it was found that only β-activity appeared as a conditioned excitation of the brain, but that no potential change in form of the so-called evoked response appeared upon conditioned stimulation.

My hearty thanks are due to Prof. K. Motokawa who furnished me with guidance and criticism in regard to this work.

References

1) Davis, P. A., J. Neurophysiol., 1939, **2**, 494.
2) Davis, H., Davis. P. A., Loomis, A. L., Harvey, E. N., & Hobart, G., J. Neurophysiol., 1939, **2**, 500.
3) Dawson, G. D., Brit. Med. Bull., 1950, **6**, 1533.
4) Dempsy, E. W., & Morison, R. S., Am. J. Physiol., 1941, **131**, 718.
5) Jarcho, L. W., J. Neurophsiol., 1949, **12**, 447.
6) Chang, H. T., J. Neurophysiol., 1950, **13**, 235.
7) Motokawa, K., Tohoku J. Exp. Med., 1949, **50**, 225.
8) Iwama, K., Tohoku J. Exp. Med., 1950, **52**, 53.
9) Forbes, A., EEG Clin. Neurophysiol., 1949, **1**, 141.
10) Bates, J. A. V., J. Physiol., 1951, **113**, 240.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

On the "Yatobyo" Exanthem (Tularemid)

By

Naoyoshi Hatchome and **Mitsuo Sato**
(八丁目 直義) (佐藤 充男)

From the Laboratory of Ohara General Hospital, Fukushima

(Received for publication, August 28, 1953)

The first clinical and epidemiological description of the disease called tularemia in modern times was made in our country in 1837 by a surgeon, Homma Soken, who was a court physician of a feudal lord of Mito district. He described this disease as "hare meat poisoning" in his textbook of surgery, Vol. 9 (Yoka-Hiroku).

In 1924 H. Ohara[1] reported for the first time in Japan scientifically on the same disease as an acute febrile disease caused by a microbe transmitted by wild rabbit. He called this disease "Yatobyo" and named the causative organism Ohara-Haga Coccus, later "Yatobyo" Bacteria.

The identity of the "Yatobyo" with tularemia, as he had anticipated, was proved in 1925 by Francis and Moore,[2] who called "Yatobyo" (tularemia in Japan) Ohara's disease, because the studies of this disease in both countries were done independently.

The exanthem which occurs in the course of tularemia was described by Francis, Parker & Spencer, Hitch & Smith,[3] Pullen & Stuart[4] and many other authors, while in Japan the exanthem which was noted by "Yatobyo" was reported only by H. Ohara in 1926 and by I. Ota[5] in 1936. Report on this case was also published by Prof. M. Ota.

Case Reports

We report here 9 instances (Table I) exhibiting the so-called "Yatobyo" exanthem among 230 cases of Yatobyo which were treated at Ohara General Hospital during the last sixteen years from Jan. 1937.

Frequency of the exanthem: According to H. Ohara, it was noted in 17 out of 239 cases (7.1%) from 1929 to 1939, and M. Sato,[6] one of the authors, observed in 7 out of 180 cases (3.9%) from 1940 to 1952 in Ohara General Hospital; Maeda (1952) reported 4 out of 33 cases (12%) at Chiba prefecture, while Miwa *et al.*[7] noted, in the same epidemic, 4 out of 10 cases (40%).

On the other hand, in America, Francis noted it in 32 out of 679 cases of tularemia (4.7%, 1928); Pullen & Stuart, in 19 out of 225 cases

(8.44%, 1944); Simpson, in 5% (1929); Kavanaugh, in 23 out of 123 cases (18.7%, 1935); Arzt, in 3 out of 15 cases (20%, 1938); Foshay, in 120 out of 600 cases (20%, 1940); Rabe & Cruse, 2 out of 81 cases (2.5%, 1950–52). H. Reich described recently that there were only two cases with exanthem out of 135 tularemia which broke out in the Turkish army at Garnison Lüleburgaz. Thus about the frequency of skin eruption of this disease the authors differ from each other in their estimations.

Sex, age and occupation: Among the nine cases we observed, six were male and three female. Their ages ranged from 22 to 59 years.

The occupation of seven among them were farming. In short, " Yatobyo " patients with exanthem reported here were mostly young male farmers, who had frequent opportunities of contact with wild rabbits and had little knowledge concerning the subject disease.

Seasonal incidence: These patients appeared chiefly in winter, seven cases in the period of October to next February, three cases of them occurred in December and the other two cases in May and July, respectively.

Most patients were infected by wild rabbits, but only one case (No. VII), which occurred in July, was caused by tick-bite on the scrotum. It responded to the seasonal incidence of all " Yatobyo " patients, of which the highest incidence occurred in the hunting season with the next greatest frequency in late spring.

Clinical classification: When these instances were classified into clinical types, the ulceroglandular type was found in four, the glandular in three, the oculoglandular and the typhoidal types in one case each. As Hitch & Smith have reported concerning tularemia, we did not observe any relation between the appearance of exanthem and the clinical cause of the disease either.

Duration from onset to eruption: The eruption appeared at the same time with the onset of the illness in some cases (No. II and IV), but it appeared commonly in the first or the second week of illness. H. Ohara or Maeda said that it appeared mainly in seven to ten days from the onset; Miwa and his co-workers said that it appeared mostly during the second week of illness.

On the other hand, Hitch & Smith reported that tularemic eruption of 29 cases appeared mostly during the second or the third week in average on the 16th day of illness. The authors reported that the shortest time which elapsed before appearance of the eruption was five days (Kerlin's case), while the longest was three months (Corn's case). Kavanaugh reported of 23 cases, and Pullen & Stuart of 15 cases, that it appeared chiefly in the second or the third week of illness.

Distribution of exanthem: This exanthem was usually noted on the

Fig. 1

Fig. 2

Fig. 1. Case VI, Erythema multiforme on neck.
Fig. 2. Case VIII, Adenopathy in left axilla, before the operation.

Fig. 3

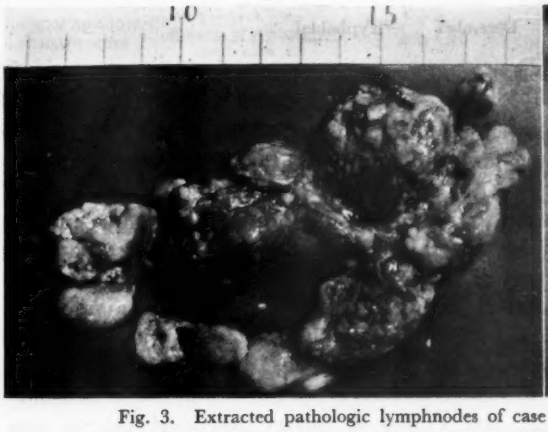


Fig. 4

Fig. 3. Extracted pathologic lymphnodes of case VIII.
Fig. 4. A week after the operation of case VIII.

upper trunk, namely on the face, the neck (Fig. I), the breast, the shoulders, the upper extremities or the hands and it was more frequently noted on the left side than on the right.

An interesting fact is that the exanthem was frequently localized on the same side as the adenopathy. But Miwa reported that it was noted

TABLE

Summary List of Cases of

No.	Sex.	Age	Occupation	Time of onset of illness (Month)	Type of illness	Portion of primary lesion	Days from onset to eruption
I	♀	36	farmer's wife	November	U. G.*	left fore-finger	5
II	♂	58	farmer	December	O. G.**	both eyes	at the same time
III	♀	59	farmer's wife	February	G.***		7
IV	♂	34	farmer	November	G.		at the same time
V	♂	34	farmer	December	U. G.	right third finger	10
VI	♂	35	labourer	May	G.		2
VII	♂	43	office worker	July	U. G.	left half of scrotum	14
VIII	♂	22	farmer	October	U. G.	left thumb	20
IX	♀	27	farmer's wife	December	Typhoidal		3

* Ulceroglandular, ** Oculoglandular, *** Glandular.

most frequently on the lower extremities and next on the forearms, the upper arms, the thigh, the forehead, the neck, the breast and the back of the body.

Hitch & Smith reported that although the eruption might be generalized or involves any one or several regions of the body, most patients had it on the upper portion of the trunk and the upper extremities, and in some instances the eruption was unilateral or at least more marked on one side. And they said that almost invariably this was the side on which the primary lesion occurred, and most patients with tularemia of the primary ophthalmic type presented a facial eruption, which was occasionally unilateral. Kavanaugh and Pullen & Stuart described that it was noted most commonly on the upper extremities, the hands or the neck on one or both sides.

Types of Eruption: The eruption was various in type and not specific; sometimes of erythema multiforme or erythema nodosum type, at others urticarial, erythematous or follicular and resembling acne vulgaris. Usually the eruption accompanied a slightly itchy or a painful feeling.

I

"Yatobyo" Exanthems

Distribution of eruption	Type of eruption	Duration of eruption (days)	Skin test (mm) 24 hours (48 hours)	Agglutination	Slide agglutination (seconds)	Bacterial culture
face, neck, breast, left hand	erythema multiforme	14	strongly positive	640×	2	(+)
neck, shoulder, left forearm, hands	erythematous	13	20×20	1280×	3	
left face	acne vulgoris		strongly positive	320×	32	
all over the body	urticarial	several days				(+)
neck, breast	follicular	16	23×25	320×	10	
left face, left neck	erythema multiforme	28	27×30 (35×40)	1280×	5	(+)
left neck, back, hands	erythema multiforme	14	15×16 (12×15)	20× 640×	70 5	(+)
face	acne vulgaris	12	35×40	1280×	5	(+)
upper and lower extremities	erythema annulare, erythema nodosum	24	20×13	80× 160×	15	(+)

It had occasionally inflammatory nature. Three cases reported by Foshay, two cases each by Hüseyin & Kemal and Arzt, and one case each by Francis, Hitch & Smith, Berde, Netoušek, Ebert and Balluch were erythema multiforme. Arzt noted one case of ecthymata, which appeared on the gluteal and the genital regions. Six cases each reported by Foshay and Pullen & Stuart, two each by Kavanaugh and Miwa, one each by Kerl and Foerster, were all of erythema nodosum type. Konrad reported one case of erythema scarlatiniforme type. Two cases reported by Ledingham & Fraser had acne vulgaris-like eruption on the back, one case by Simpson had it on the neck too, more than 17 cases by Pullen & Stuart had herpes labialis type, and Francis described one case of "extreme herpes."

The original cause of these eruptions was, as H. Ohara and I. Ota reported concerning the first case of our collections, presumed to be allergic in nature. It was presumed that the eruption was of septic-pyemic nature, which occurred as a result of lymphatic and hematogenic spreading of bacterial toxic factors.

Duration of the eruption: The average duration of the eruption was

19.5 days; mostly it disappeared leaving no trace in one or two weeks after extirpation of the pathologic lymph nodes (Fig. 2, 3 and 4) and in four cases after injection of 0.5 g. of streptomycin every day (No. VI, VII, VIII and IX). Only one case, No. VII, showed pigmentation after the disappearance of the eruption.

Miwa reported that the eruption disappeared in 3 to 7 weeks after the appearance. Hitch & Smith reported that the shortest duration of the eruption was two days, while the longest was in a case reported by Mc Gray, in which there were recurrent manifestations for over 113 days, and the average duration of the eruption was twenty one and nine tenths days.

As our report showed, Pullen & Stuart also described the eruption as persisting for several days to about three weeks. But they described that doubt existed as to the etiology of the four patients in erythema nodosum type, because sulfonamides were being administered when the skin manifestations first became appearent, and in each of these cases the skin eruption subsided within four days after the sulfonamides administration was discontinued.

Specific examinations: 1) Intradermal test. This examination is the most reliable diagnostic method for this deisease, the reaction occurring in a specific manner in two or three days after the onset of the illness. Wherry advocated at first this specific reaction in 1917 and later in 1932 Foshay[8] made bacterial protein antigen for this skin test for tularemia, while in Japan H. Ohara, Kobayashi, Hiwatashi and T. Ota reported the skin test for "Yatobyo," in which bacterial suspension was used as antigen.

We made an antigen by the following method: A 0.01% bacterial suspension of heat-killed bacterium tularense was made from Ebina strain, to which 0.5% carbol was added. 0.1 cc. of this fluid was injected intradermally in the forearm. This test was done in 8 out of 9 cases here reported, all of which showed extreme positive reaction; the local portion became reddish and showed swelling in the size of 2 to 4 or 5 cm. in diameter 12 hours after the injection. After 24 hours it became edematous and blistered and was accompanied by a slight pain or a fairly itchy feeling, but after 72 hours the lesions began to fade slowly and pigmentation was left at the spot of injection.

2) Serum-agglutination. A specific agglutinin is produced in the serum of "Yatobyo" patients, and so serum agglutination too is useful, but it usually becomes positive after eigh or ten days, sometimes in the second week of the illness. So this test can not be used as a method of early diagnosis. The titre rises generally to the highest level in the 3rd or 4th week, thereafter it falls down gradually and the agglutinin of lower

titre persists for the length of about twenty or thirty years. We made a formolized (0.5%) suspension of bact. tularense (5 mg/cc), by which eight out of nine cases reported here were tested for agglutination. Case VII showed a level of 1:20 on the 11th day, case IV showed a level of 1:80 on the 12th day of illness and the titre of the later case rose on to 1:1280 after about a month.

3) Slide agglutination. A drop of the serum of hemolyzed blood of the patient was put on a slide glass, to which a drop of the bacterial suspension was mixed and then the agglutination, which might occur within about a minute, was examined. We tested all cases and the average time of slide agglutinations was 18 seconds. A case, in which this test occurred in 70 seconds on the 11th day of the illness, showed positive result in 5 seconds after about a month (case VII).

Summary

Nine cases exhibiting cutaneous eruptions among 230 cases of "Yatobyo" (Tularemia in Japan) at Ohara General Hospital during the last sixteen years from Jan., 1937 to Dec., 1952 were studied.

Most of them were young farmers (6 male and 3 female) from 20 to 30 years of age, who had many chances of contact with wild rabbits in the hunting season, (from November to next February), but only one case, which occurred in July, was caused by tick-bite on the scrotum.

The eruption developed chiefly during the first or second week of the illness, but in two cases, it developed at the same time with the onset of the disease. The sites of predilection are the face, the neck, the breast and the upper extremities, i.e. parts in the upper half of the body. Moreover, it is an interesting fact that the exanthem was frequently localized at the same side as the adenopathy.

The eruption was various in type and not specific; sometimes of erythema multiforme or erythema nodosume type, at others urticarial, erythematous or follicular and resembling acne vulgaris. Usually the eruption accompanied fairly itchy or painful feelings.

It seemed to be an allergic exanthem, which disappeared with no trace in two or three weeks.

The skin test by heat-killed bacterial suspension was positive in eight cases, and the serum- and slide agglutination tests were positive in all cases. The antigen used was made from bact. tularense, Ebina strain, which was isolated from "Yatobyo" patients in our laboratory.

We express our deepest gratitude to Dr. Minor Itô, Professor of Tohoku University, for kindly revising this report, and to Dr. S. Ohara, Director of Ohara General Hospital, for his guidance in preparing it.

References

1) Ohara, Igaku Soran, 1944, Vol. I, Kanehara Shoten, Tokyo.
2) Francis & Moore, J.A.M.A., 1928, **91**, 1155.
3) Hitch & Smith, Arch. Derm. & Syph., 1938, **38**, 859.
4) Pullen & Stuart, J. A. M. A., 1945, **129**, 495.
5) Ota, Jap. J. Derm. & Vener. (Jap.), 1937, **42**, 127.
6) Sato, Jap. J. Sanitary Zoology (Jap.), 1952, **3**, 63.
7) Miwa *et al.*, Jap. J. Infect. Dis. (Jap.), 1951, **24**, 158; *ibid*, 1952, **25**, 171.
8) Foshay, J. Infect. Dis., 1932, **51**, 286.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Studies on Brain Stem

VII. On the Position of the Abducens Nucleus in Human Embryos, Viewed Ontogenetically

By

Shigeaki Yamamoto, Mariko Komatsu, Mitsuyoshi Oikawa
(山本 重亮) (小松 満利子) (及川 光榮)
and **Toshiaki Konno**
(今野 利秋)

From the Anatomical Laboratory of Prof. H. Seto, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, September 3, 1953)

The authors[1)2)3)] have reported previously on their serial studies on the facial nuclei, with criticism on the neurobiotaxis theory of A. Kappers. In this paper, we wish to reconsider the said theory with reference to the abducens nucleus.

Kappers[4)5)] contends that the abducens nucleus located more ventrally in phylogenetically lower animals is attracted by the stimulus of the medial longitudinal fasciculus to a more dorsal position in higher animals. He also supported the ontogenetical studies of Gaskell and Carpenter who stated that the abducens nucleus in birds and mammals shifts from their ventral position in embryonic stage to a more dorsal position following its development, to emphasize the veracity of his proposition.

Fuse,[6)7)] however, in his minute study on the abducens nucleus, has announced that the nucleus does not follow such a simple phylogenetical rule of displacement as Kappers would have it, and ontogenetically also it shows no trace of movement, but is stationary at the same position since the earliest stage of embryonic life, at least in man.

The authors of this paper have undertaken to reconsider this problem factually, through a study of sets of Pal-carmine stained transverse sections of brains of human embryos of every month between the second and tenth months, as follows.

Experimental

Findings

Owing to the undifferentiated state of the various nuclei in the brain, the position of the abducens nucleus in a second month human embryo could not be located with any precision.

The position of the abducens nucleus was clearly determinable in the sections of embryos of three months and more in age. In the third month embryo already, the abducens nucleus occupied the same dorsal position as in a tenth month embryo, or for that matter, as in adult brains.

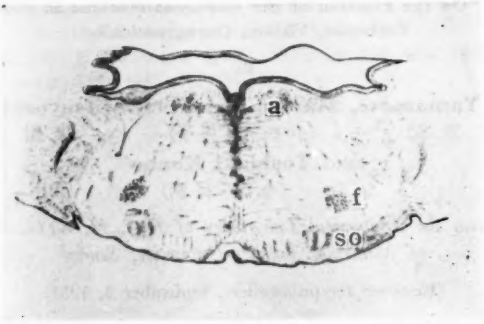


Fig. 1. Transversal section at the level of abducens nucleus in third month human embryo, Pal-carmine staining, lwo power view. a abducens nucleus; f facial nucleus; so superior olive.

In the third month embryo, the nerve cells are discernible as slightly red staining small bodies, but since the nerve fibres are as yet unmyelinated, the medial longitudinal fasciculus was not observable in our sections. This would indicate that, since the medial longitudinal fasciculus is as yet unmyelinated and in a state of imperfect functionality, the abducens nucleus should be located more ventrally, if we follow the Kappers' theory. The reports of Gaskell and Carpenter also would indicate a more ventral position for the abducens nucleus in a third month embryo, but as stated above, the nucleus was found in the same position occupied by it in adult brains, according to our observation.

In all the embryos between four and ten months in age, the abducens nucleus occupied exactly the same position as in the third month embryo, developing and maturing there without any trace of displacement, notwithstanding the development of cell elements and fibre elements in the interim. This observation is in strict agreement with that by Fuse[6)7)] and apparently contradicts the proposition of Kappers.

Moreover, through all the months between the third and the tenth of the embryonic life, the abducens nucleus is found in a dorsolateral position to the medial longitudinal fasciculus, growing in a manner as if it pushed up the floor of the fourth ventricle of the brain. If the abducens nucleus were attracted toward the medial longitudinal fasciculus by its stimulus from a more ventral position, as Kappers' theory would presume,

Fig. 2

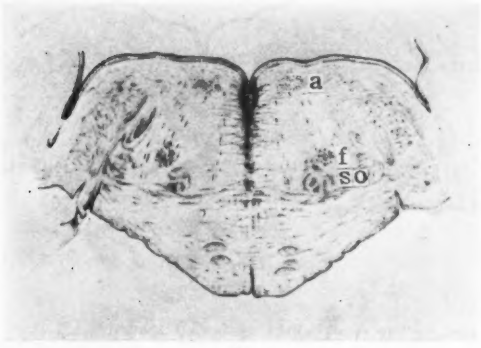


Fig. 3

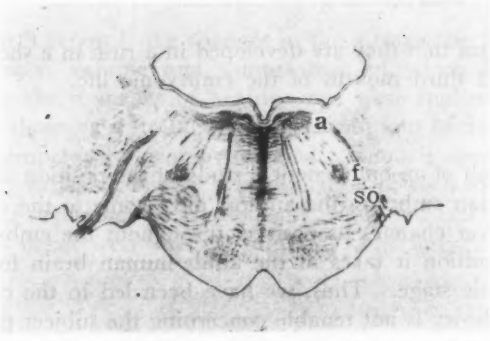


Fig. 2. Transversal section at the level of abducens nucleus in fourth month human embryo, Pal-carmine staining, low power view. a abducens nucleus; f facial nucleus; so superior olive.

Fig. 3. Transversal section at the level of abducens nucleus in fifth month human embryo, Pal-carmine staining, low power view. a abducens nucleus; f facial nucleus; so superior olive.

the laterodorsal position of the nucleus relative to the fasciculus is very difficult to explain.

Fuse[6][7] has divided the abducens nucleus into the two parts of that buried in the reticular substance and of that above the reticular substance and jutting into the floor of the fourth ventricle and pointed out the difference in the cytoarchitecture of the two parts. In our sections, the distinction between the two parts was well established in the embryos older than three months. It is of some interest that the various nuclei in the brain of an embryo are quite clearly distinguishable in the third month, although it is impossible to distinguish them from each other in the second

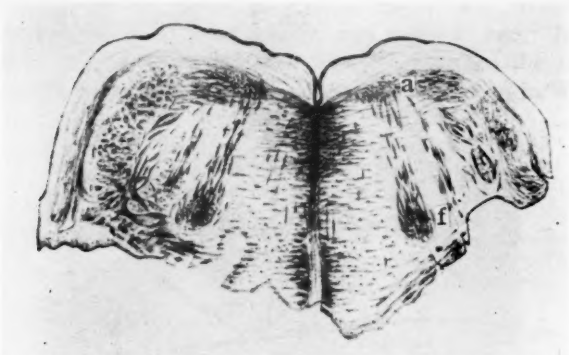


Fig. 4. Transversal section at the level of abducens nucleus in tenth month human embryo, Pal-carmine staining, low power view. a abducens nucleus; f facial nucleus.

month. It seems that they are developed in a rush in a short time within the second and third months of the embryonic life.

Conclusion

As the result of an ontogenetical study of the position of the abducens nucleus in human embryos, the authors have come to the conviction that the nucleus never changes its position throughout the embryonic life, occupying the position it takes in the adult human brain from the outset of the embryonic stage. Thus, we have been led to the conclusion that the Kappers' theory is not tenable concerning the subject problem either.

References

1) Yamamoto, Tohoku J. Exp. Med., 1952, **56**, 331.
2) Yamamoto, *ibid.*, 1952, **56**, 339.
3) Yamamoto & Komatsu, *ibid.*, 1953, **57**, 359.
4) Kappers, Neurol. Zentralbl., 1907, **26**, 834.
5) Kappers & Vogt, *ibid.*, 1908, **27**, 958.
6) Fuse, *ibid.*, 1911, **30**, 178.
7) Fuse, Arb. hirnanat. Inst. Zürich, 1912, **6**, 405.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Studies on the Temperature of the Gastrointestinal Tract

Tenth Report

Influence of Ingesting Various Solutions on the Intragastric Temperature

By

Mitsuo Ohara
(大原 光雄)

From the Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai

(Received for publication, September 10, 1953)

In the fourth report,[1] the changes in the intragastric temperature by introducing water, volume and temperature of which were variously combined, into the stomachs of healthy men were studied by Masuda.[1] In this report, those by introducing various solutions of durgs of constant volume and temperature are investigated. Though several studies[2]-[4] were presented, in which one or a combination of such foods and drinks as bread, meat, vegetable, ice cream, milk, soup and coffee was ingested, those in which drugs were used in solution, are scanty.[5]

Experimental

Methods and Materials

Because, as reported previously,[1] the stomach showed the most specific behaviour when 250 cc. of 15–16°C water was introduced into it, it seemed to react most sensitively to such volume and temperature of water. Accordingly in this study, the volume and temperature of the solutions to be introduced were made in the same way as mentioned above. The intragastric temperature was measured in a half-supine position by the thermometer,[6] which was introduced into the stomach at 60 cm. from the incisor in a fasting state. After it became almost constant, 2% alcohol, 0.05 and 0.1% hydrochloric acid, 0.9% solution of sodium chloride, 0.07% of caffeine, 0.016% of banthine, 2 and 4% of sodium bicarbonate and 1% suspension of anion exchange resin (stomaresin) in the volume and temperature as before, were introduced into the stomach through the Miller-Abbott's tube of the thermometer in about 75 seconds. For 30 minutes thereafter it was continuously measured. As a control water was introduced as before. Simultaneously 5 cc. of the gastric content

was aspirated at each 10 minutes and the free and total acidities were determined as in the fifth report.[7] For each of the above stated 9 solutions and water 15 individuals, totaling 150 healthy adults who had no previous sign of gastrointestinal disorders, were examined. The room temperature was ranged between 12 and 24°C.

Results

As soon as the water and solutions began to be introduced into the stomach, the intragastric temperature dropped abruptly and continued to drop during the introducing. Immediately or at least about 10 seconds after the introducing it reached the maximum drop, though it never reached the temperature of the water and solutions. Soon thereafter it began to recover at first rapidly then progressively slowly as shown in Table I. In case of water, these changes were expressed by an exponential function of time after the introducing as shown in Fig. 1. The empirical formula shown in Table II is obtained from 15 cases of the present experiment and do not differ from that of the previous one.[1]

Next in cases of caffeine and banthine solutions (group I), the maximum drops in the intragastric temperature immediately following their introducings did not differ significantly from that of water. However, the changes thereafter observed from the values of 2, 10, 18 and 26 minutes after the introducing differed significantly. The empirical formulas are also respectively expressed by an exponential function as given in Table II and their slopes (regression coefficients) in the semilogarithmic table (Fig. 1) are significantly smaller than that of water. As in these changes individual variations appeared considerably, the ranges of the changes overlapped mutually in part. However, the means were significantly different as mentioned before except between caffeine and banthine.

In cases of 0.05 and 0.1% HCl, 2 and 4% $NaHCO_3$ solutions and 1% suspension of stomaresin (group II), the curves of change in a semilogarithmic table respectively run upward for the first 4 to 6 minutes following their introducings and then recover almost linearly as shown in Fig. 2. Accordingly the empirical formulas are, as given in Table II, respectively expressed by two exponential functions, the first of which represents the linear portion and the second the convex portion. In $NaHCO_3$ the changes both before and after the point of 4 minutes after the introducing differed somewhat according to concentration. In the concentrated solution the above mentioned convexity before the point was more remarkable and the slope of the linear portion after the point was smaller than in the dilute one. Namely the more concentrated the solution, the more manifest its characteristic actions both before and after

TABLE I

The Maximum (Max.), Minimum (Min.), Mean and Standard Deviation (St. d.) of Each Change in the Intragastric Temperature (I.G.T.) before and after Introducing Respectively 250 cc. of Water, Solutions and Suspension

Fluid	Temp. of fluid °C	Time for introd. sec.	I.G.T. before introd. °C		Change in I.G.T. (°C) Time (min.) after administration 0	2	4	6	10	18	26	30
Water	16.0	85	37.8	Max.	−20.6	−15.8	−10.2	−7.0	−3.4	−0.9	−0.3	−0.2
	15.0	65	36.7	Min.	−12.9	− 7.9	− 4.6	−2.5	−0.8	−0.1	0	0
	15.4	74.7	37.23	Mean	−17.82	−11.10	− 7.22	−4.66	−2.08	−0.43	−0.11	−0.04
	0.5	8.2	0.30	St. d.	1.83	2.06	1.75	1.29	0.73	0.28	0.10	0.08
0.07% Caffeine	16.0	90	38.2	Max.	−21.6	−14.6	−11.8	−9.7	−6.0	−2.5	−1.2	−0.8
	15.0	70	37.5	Min.	−16.6	− 8.8	− 6.0	−4.4	−1.9	−0.5	−0.1	0
	15.4	75.0	37.75	Mean	−19.05	−12.44	− 8.88	−6.83	−3.91	−1.23	−0.41	−0.22
	0.5	4.8	0.19	St. d.	1.22	1.76	1.61	1.71	1.26	0.71	0.34	0.21
0.016% Banthine	16.0	80	38.2	Max.	−20.0	−15.1	−12.3	−9.7	−5.7	−1.9	−0.5	−0.3
	15.0	65	36.7	Min.	−14.2	− 8.2	− 4.1	−3.6	−2.0	−0.5	−0.1	−0.1
	15.1	74.7	37.50	Mean	−18.15	−11.43	− 8.18	−6.20	−3.50	−0.99	−0.29	−0.16
	0.3	4.6	0.37	St. d.	1.82	1.85	2.32	1.84	1.18	0.40	0.13	0.08
0.05% HCl	16.0	85	37.9	Max.	−20.6	−13.9	−10.4	−8.2	−6.7	−3.4	−1.1	−1.0
	15.0	70	37.1	Min.	−16.4	− 8.0	− 5.9	−4.2	−2.3	−0.6	−0.1	−0.1
	15.4	74.7	37.56	Mean	−18.59	−10.90	− 7.96	−5.98	−3.73	−1.38	−0.53	−0.35
	0.3	4.6	0.27	St. d.	1.03	1.50	1.31	1.24	1.21	0.71	0.36	0.27
0.1% HCl	16.0	85	38.1	Max.	−20.3	−13.0	−10.0	−7.9	−5.5	−2.7	−1.5	−0.9
	15.0	65	36.7	Min.	−16.0	− 9.0	− 5.9	−4.7	−2.5	−0.5	−0.2	−0.1
	15.5	75.0	37.47	Mean	−18.73	−11.23	− 8.16	−6.31	−3.88	−1.58	−0.67	−0.42
	0.4	4.8	0.58	St. d.	1.16	1.27	1.28	0.92	0.89	0.75	0.39	0.23
2% $NaHCO_3$	16.0	85	38.0	Max.	−19.9	−15.3	−11.7	−9.4	−6.0	−2.6	−0.7	−0.4
	15.0	65	37.3	Min.	−15.9	− 6.1	− 2.7	−1.9	−0.5	−0.1	0	0
	15.4	75.3	37.58	Mean	−18.03	−11.05	− 7.37	−5.07	−2.65	−0.76	−0.23	−0.10
	0.3	5.3	0.20	St. d.	1.28	2.61	2.63	2.44	1.56	0.65	0.20	0.12
4% $NaHCO_3$	16.0	85	38.0	Max.	−21.0	−14.6	−10.8	−8.4	−6.0	−2.9	−1.7	−1.3
	15.0	65	37.0	Min.	−14.1	− 3.5	− 2.5	−2.0	−1.4	−0.5	−0.2	−0.1
	15.1	75.3	37.46	Mean	−18.55	− 9.24	− 6.83	−5.17	−3.29	−1.35	−0.57	−0.41
	0.2	5.6	0.28	St. d.	1.89	3.04	2.45	1.88	1.46	0.81	0.48	0.37
1% Stoma-resin	16.0	120	38.2	Max.	−21.0	−15.5	−11.1	−9.2	−6.3	−2.0	−0.8	−0.5
	15.0	80	37.1	Min.	−14.3	− 8.7	− 6.1	−3.8	−1.8	−0.4	0	0
	15.5	107.0	37.68	Mean	−17.51	−11.16	− 8.19	−6.06	−3.57	−1.11	−0.41	−0.26
	0.5	12.1	0.35	St. d.	2.16	1.82	1.60	1.45	1.18	0.54	0.23	0.16
2% Alcohol	16.0	80	38.0	Max.	−20.6	−14.1	−10.3	−8.1	−5.1	−2.3	−0.9	−0.6
	15.0	65	37.3	Min.	−16.3	− 6.6	− 2.5	−1.6	−0.7	−0.1	0	0
	15.2	74.7	37.62	Mean	−18.99	−10.80	− 7.21	−5.21	−2.75	−0.81	−0.28	−0.21
	0.4	3.9	0.24	St. d.	1.11	2.39	2.42	2.17	1.42	0.68	0.28	0.19
0.9% NaCl	16.0	85	37.9	Max.	−20.5	−13.7	−10.3	−7.9	−5.0	−2.7	−0.8	−0.4
	15.0	70	36.7	Min.	−14.0	− 8.6	− 4.5	−2.8	−1.6	−0.4	−0.1	0
	15.5	75.0	37.48	Mean	−18.15	−10.56	− 7.47	−5.41	−3.27	−1.16	−0.37	−0.17
	0.4	3.2	0.28	St. d.	1.52	1.66	1.63	1.58	0.94	0.58	0.19	0.11

the point, and consequently the curves of 2 and 4% solutions crossed mutually. In HCl the changes were similar regardless of its concentration throughout 30 minutes and their means were larger according to its con-

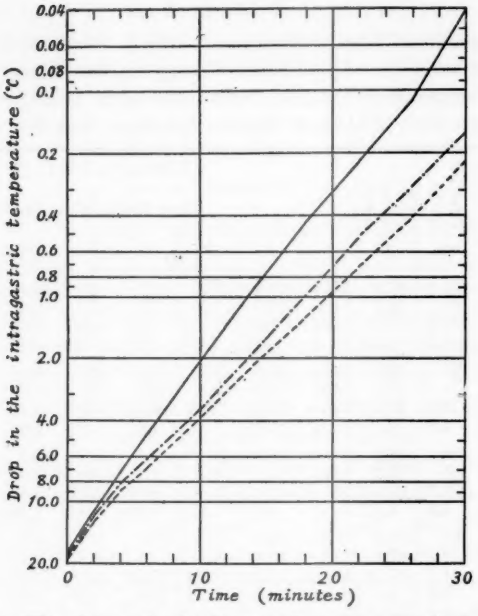


Fig. 1. The changes in the intragastric temperature (in logarithmic scale) after introducing water (————), 0.07% caffeine (- - - - - -) and 0.016% banthine solutions (— — — — — — —). Minus sign in the temperature scale is omitted.

centration, though no significant difference was noted. In stomaresin, though the time for the introducing was longer than the others (Table I), the change was similar to that of 2% $NaHCO_3$ solution.

In cases of 2% alcohol and 0.9% NaCl solution (group III), their curves on the semilogarithmic table (Fig. 3) run convexly upward for the first 6 minutes and thereafter recover almost linearly up to 22 minutes after their introducings as in group II. Thereafter, however, in alcohol the curve again extended convexly upward and in NaCl, on the contrary, concavely. Consequently the empirical formulas are, as shown in Table II, expressed by three exponential functions, the first and the second of which represent the same portions as in group II and the third the convex (+sign) or concave (—sign) portion. In alcohol individual variations were considerably great.

The gastric free acidity was increased by HCl, caffeine, alcohol and NaCl, and decreased by $NaHCO_3$ and stomaresin. The changes by banthine and water were not marked (Fig. 4). In each drug, there was no constant relation between the acidity and the intragastric temperature.

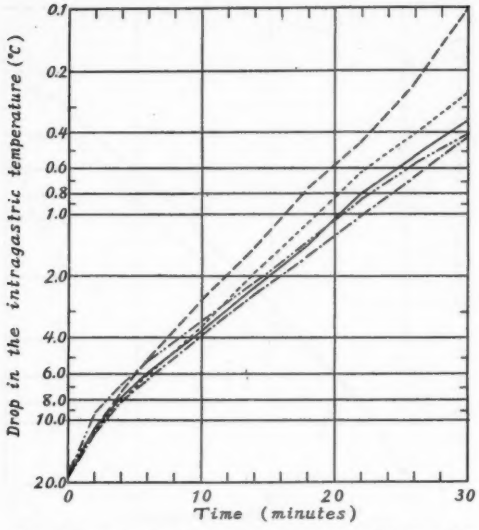


Fig. 2. The changes in the intragastric temperature (in logarithmic scale) after introducing 2% (— — — — —) and 4% solutions (— - — - — - —) of $NaHCO_3$, 0.05% (————) and 0.1% (— - — - — - — - —) of HCl and 1% suspension of stomaresin (- - - - - - - -). Minus sign in the temperature scale is omitted.

TABLE II

Empirical Formulas of Changes in the Intragastric Temperature after Introducing Water, Solutions and Suspension

Water :	$Y = -16.06\ e^{-0.200t}$
0.07% Caffeine :	$Y = -16.85\ e^{-0.145t}$
0.016% Banthine :	$Y = -16.24\ e^{-0.155t}$
0.05% HCl :	$Y = -(12.12\ e^{-0.120t} + 5.99\ e^{-0.661t})$
0.1% HCl :	$Y = -(11.58\ e^{-0.110t} + 6.26\ e^{-0.517t})$
2% $NaHCO_3$:	$Y = -(13.39\ e^{-0.159t} + 4.86\ e^{-0.702t})$
4% $NaHCO_3$:	$Y = -(9.62\ e^{-0.108t} + 7.76\ e^{-0.684t})$
1% Stomaresin :	$Y = -(12.88\ e^{-0.133t} + 4.04\ e^{-0.469t})$
2% Alcohol :	$Y = -(13.08\ e^{-0.155t} + 5.74\ e^{-0.811t} + 0.001\ e^{0.160t})$
0.9% NaCl :	$Y = -(11.74\ e^{-0.128t} + 6.16\ e^{-0.675t} - 0.001\ e^{0.148t})$

Remark : Y°C is the change in the intragastric temperature t minutes after introducing.

DISCUSSION

Volume and temperature of each solution introduced into the stomach were made just the same as the control, i.e., water. Speed for the introducing was also made equal except for stomaresin. Therefore, if there

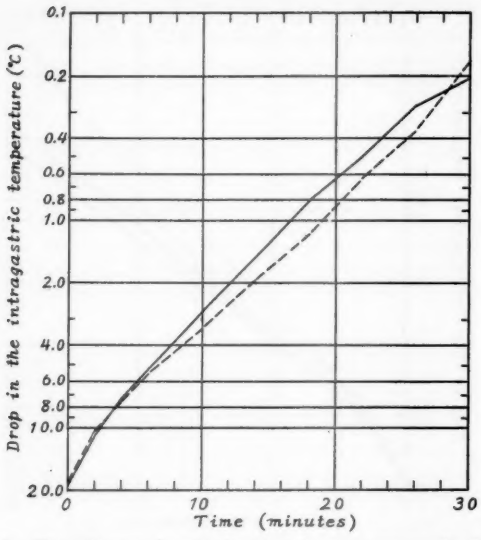


Fig. 3. The changes in the intragastric temperature (in logarithmic scale) after introducing 2% alcohol (————) and 0.9% solution of NaCl (- - - - - - - -). Minus sign in the temperature scale is omitted.

is any difference between the change in the intragastric temperature by the solution and that by water, the cause is ascribed to the action of the drug upon the stomach.

Among the maximum drops in the intragastric temperature following the introducings of water and solutions, significant differences were noted only exceptionally—only in 3 among the 45 combinations. Consequently it is concluded that the drugs have no effect upon the change immediately following the introducing. The maximum drop is essentially decided by the temperature of the introduced fluids, as reported previously.[1)]

The curves of recovery in the intragastric temperature thereafter were all expressed by one, two or three exponential functions of time after the introducing and were divided into three groups according to the number. In case of water the curve was expressed by an exponential function in the present experiment. It was also true over the ranges both from 50 to 450 cc. and 2 to 47°C as reported previously.[1)] Therefore, the fact that in groups II and III the curves were expressed by not one exponential function but two or three, is due to the actions of the drugs. Moreover, the curves of group I differed somewhat from that of water and showed a tendency to approach group II (Fig. 1). It is supposed that if the solutions are more concentrated, group I belongs in

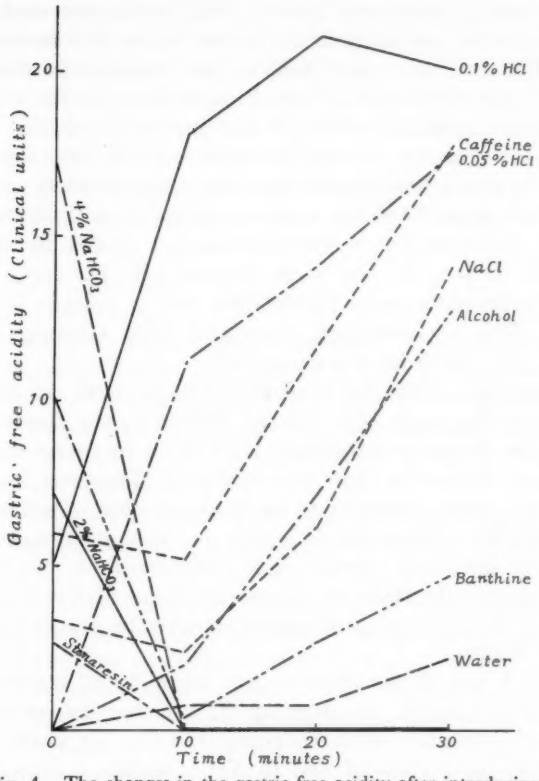


Fig. 4. The changes in the gastric free acidity after introducing water, solutions and suspension.

group II. The most remarkable difference between the changes by the various solutions and that by water, was the convexity in the curves of recovery in a semilogarithmic table during the first several minutes following their introducings. It is known that water is very rapidly discharged from the stomach into the duodenum.[8)9)] Moreover, that hydrochloric acid as well as some other substances, even various irritants, relax the pyloric sphincter, is also known.[10)] Accordingly both water and solutions introduced into the stomach flow rapidly into the duodenum. However, the ejected gastric contents in the duodenum stimulate the duodenal mucosa and then duodenopyloric or enterogastric reflex, which checks gastric discharge, takes place.[8)9)11)12)] Therefore, solution flowing in the duodenum inhibits the further flow from the stomach contrary to water, and the change in the intragastric temperature may show such

specific behaviour as mentioned above. This is characterized in a semilogarithmic table by convexity of the curve for the first several minutes. As mentioned before, the second term of the empirical formula expresses the convexity, the magnitude of which depends upon the coefficient as well as the power (absolute value) of the empirical formula. Moreover, in $NaHCO_3$ it is probable that the space around the thermometer which is placed in the stomach at 60 cm. from the incisor is filled with carbonic acid gas formed from $NaHCO_3$ in the presence of acid in the stomach. Consequently the recovery of the thermometer cooled by the solution will be rapid. It may be one of the reasons why the curve of the 4% solution is exceptionally rapid for the first several minutes following the introducing. This is interesting compared with Cannon's view that $NaHCO_3$ retards exit from the stomach.[8]

Thereafter, the curves in a semilogarithmic table in the solutions recover linearly the same as in water. However, the slopes which are characterized by the power (absolute value) of the first term of the empirical formula are all smaller than that of water. Moreover, the slope becomes inversely small according to the concentration of solution in both $NaHCO_3$ and HCl. Therefore, it is also due to the actions of drug that the recovery in the linear protion in a semilogarithmic table is delayed. This means apparently retarded gastric emptying and is chiefly due to enterogastric reflex and changed gastric motility by the chemical stimulation of the drugs.

In groups I and II the recovery was linear by 30 minutes after the introducing (Figs. 1 and 2), but in group III a further change was observed at about 22 minutes after the introducing (Fig. 3). In alcohol the speed of recovery was more reduced and in NaCl, on the contrary, more accerelated. These changes are characterized by the third additional term of empirical formula, which is a plus sign in the former and a minus one in the latter. This is, as it were, the after-effect of drug and shows that gastric motility changes again with some interval after the introducing. This may be caused not only by the peripheral actions of drug, but also by the central actions. However, their explanations cannot be given without further experiments.

From the above discussion, it is certain that the drugs display their actions on the change in the intragastric temperature in three different forms: the first is the immediate action, the second the intermediate one, and the third the after-effect, which may at times not appear.

Thiessen and Snell,[5] though they performed experiments about alcohol, reported that the intragastric temperature rose on account of hygroscopic chemical reaction of the alcohol, when 50 cc. of 10% alcohol the temperature of which was equal to that in the stomach was ingested.

However, in our experiment this was not observed, because the temperature of the alcohol was 15–16°C.

In the experiments the various drugs, which were classified in gastric secretogogue (caffeine and alcohol), antacid ($NaHCO_3$ and stomaresin), neutral salt (NaCl), autonomic blocking drug (banthine) and acid (HCl), were introduced into the stomach. However, among the changes in the intragastric temperature by these drugs the peculiarity, which characterized distinctly the above classification, was not noticed, contrary to the changes in the gastric acidity.

Summary

Responses of the intragastric temperature to introducing 250 cc. of 15–16°C water, 2% alcohol, 0.05 and 0.1% HCl, 0.9% solution of NaCl, 0.07% of caffeine, 0.016% of banthine, 2 and 4% of $NaHCO_3$, and 1% suspension of stomaresin respectively into the stomachs of healthy men, were studied simultaneously with those in the gastric acidity; and the following facts were confirmed.

1. The intragastric temperature reaches the maximum change immediately following introducing of the water, solution or suspension and then recovers.

2. The drugs have no effect upon the maximum change.

3. The drugs display their actions on the recovery in three different forms: the first is the immediate action, the second the intermediate one, and the third the after-effect, which may at times not appear.

4. Accordingly, the change in the intragastric temperature by introducing of the solution or suspension is, as a rule, expressed by two or three exponential functions.

5. The drugs do not exhibit their chemical or pharmacological characteristics, such as gastric secretogogue, antacid and so on, in the change in the intragastric temperature, contrary to that in the gastric acidity.

References

1) Masuda, Tohoku J. Exp. Med., 1953, **57**, 119.
2) Stengel & Hopkins, Amer. J. Med. Sci., 1917, **153**, 101.
3) Hepburn, Eberhard, Ricketts & Rieger, Arch. Int. Med., 1933, **52**, 603.
4) Eberhard, Rev. Gastroent., 1940, **7**, 133.
5) Thiessen & Snell, Amer. J. Physiol., 1933, **105**, 665.
6) Masuda, Tohoku J. Exp. Med., 1951, **54**, 355.
7) Masuda, Ohara & Katsura, *ibid.*, 1953, **57**, 129.
8) Cannon, Amer. J. Physiol., 1907, **20**, 283.
9) Portis, Diseases of the Digestive System, Lea & Febiger, Philadelphia, 2nd Ed., 1946, p. 116.

10) Shay & Gershon-Cohen, Surg., Gynec. and Obst., 1934, **58**, 935.
11) Thomas, Crider & Mogan, Amer. J. Physiol., 1934, **108**, 683.
12) Thomas & Crider, Virginia Med. Month., 1937, **64**, 181.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Studies on the Temperature of the Gastrointestinal Tract

Eleventh Report

A New Thermometer for the Gastrointestinal Temperature Measurement

By

Hisayuki Masuda, Mitsuo Ohara and **Shigeaki Katsura**
(増田久之) (大原光雄) (桂 重暉)

From the Medical Clinic of Prof. T. Kurokawa,
Tohoku University, Sendai

(Received for publication, September 10, 1953)

Hitherto in our studies, as an intragastric thermometer, only the platinum resistance thermometer, the details of which were given in the first report,[1] has been employed. Recently a thermistor, or thermally sensitive resistor, has been applied to thermometry[2][3] and by using it we obtained satisfactory results in measuring the intragastric and rectal temperatures of rabbits.[4] In this report, a thermistor thermometer to measure the temperatures not only in the esophagus and stomach but also in the intestine of the human body and the comparison of it with the platinum thermometer are presented.

Experimental

Materials and Methods

The thermistor thermometer constructed by us consists of two parts as the platinum resistance thermometer[1]: a thermometer (Fig. 1) and an indicator. In Fig. 1, A and B are respectively a hollow glass cylinder, 7.5×3.5 mm., in which a small bead type thermistor is enclosed. C is the Miller-Abbott's tube; one tube serves for lead wires from the thermistors (between the terminal T_1 and both A and B) and the other for aspiration of contents in the part of the gastrointestinal tract, into which the glass cylinder A is introduced, from the opening E. The tube is visible at fluoroscopy. The glass cylinder A is attached to the top of the tube and B is 25 cm. apart from A. Two lead wires from A run through the glass cylinder B and one of the two is also used for that of B. Fig. 2 is the electrical circuit of the thermometer above described and by the switch S one of the two thermistors is independently connected to the indicator

through the terminal T_2. The indicator is essentially the same as that of the platinum thermometer. As a thermistor has high negative temperature coefficient, a load for the Wheatstone's bridge of the indicator is controlled by current and not by voltage. The current loaded was made 50 μA, because self-heating of the thermistor by the stronger current disturbed the characteristic resistance-temperature relation. The method of operating this thermometer and its calibration to temperature are the same as the platinum one and 0.1°C corresponds to 4 readings of the galvanometer scale of the indicator.

To compare this thermometer with the platinum one, both thermometers and the standard mercury one were together submerged into the water bath, water in which was being stirred up constantly to make its temperature uniform. After the three thermometers showed the constant temperature reading (20–25°C), hot water was poured into the water bath intermittently with an interval of 30 to 60 seconds for 12 minutes (intermittent experiment). In each interval the temperature in the water bath was measured simultaneously with the three thermometers, the readings of which were recorded by each observer. Next, after the three thermometers showed the constant temperature reading (35–40°C), cold water was poured into the water bath continuously through a small rubber tube for 5 minutes (constant experiment). At each 20 seconds the readings of the three thermometers were simultaneously observed and recorded as before.

The platinum thermometer has been somewhat improved since the fourth report.[5] Namely, the electrical resistance is made smaller (ca. 40 ohms) and 4 readings of the galvanometer scale correspond to 0.1°C.

Results

A part of the resistance-temperature relation of the thermistor thermometer is given in Table I and Fig. 1, with that of the platinum one. Both the electrical resistance and the rate of its change per degree of temperature are much higher in the thermistor than in the platinum. Obviously in Fig. 1, though the relation is linear in the platinum, it is not so in the thermistor. That of the latter is expressed as follow: logarithm of the resistance is a function of the inverse absolute temperature.

The data of the intermittent and the continuous experiments are shown in Tables II and III respectively. In the thermometry of both ascending and descending temperature gradients, the thermistor as well as the platinum thermometers showed satisfactory results comparing to the standard mercury one and difference between them was scarcely noticed. A time lag was not found in the experiments.

Moreover, the thermistor thermometer had long-time stability which

TABLE I

Temperature-Resistance Relation of Thermistor and Platinum Thermometers

Temp. °C	Resistance		
	Thermistor thermometer ($\times 10^3$ ohms)		Platinum thermometer ohms
	A	B	
10	157.0	156.7	39.43
15	123.0	123.3	40.20
20	97.50	98.18	40.98
25	77.63	78.70	41.77
30	62.24	63.24	42.56
35	50.46	51.64	43.37
40	41.11	42.17	44.18
45	33.81	34.76	45.06

Fig. 1

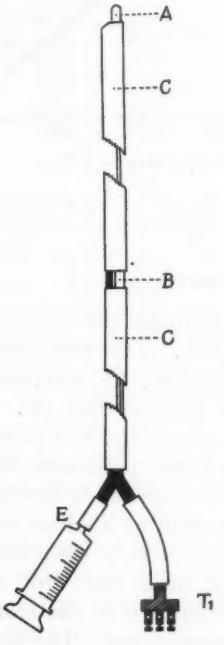


Fig. 2

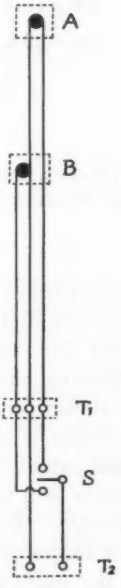


Fig. 1. Thermometer.
Fig. 2. Electrical circuit of the thermometer.

was proved by its calibration performed at every one or two weeks. It was almost equal to that of the platinum thermometer.

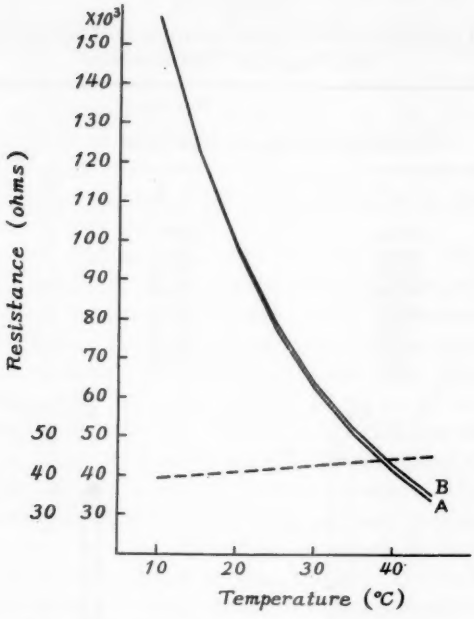


Fig. 3. Resistance-temperature relations of thermistor (————) and platinum thermometers (- - - - - - - -).

Discussion

As both the thermistor and the platinum thermometers are resistance ones, their general principle is the same. The differences between them are the shape and size, the heat capacity, the electrical resistance, the temperature coefficient, the stability, and the cost, etc.

As in the platinum thermometer a long and fine platinum wire which is coiled on a cylinder is used as its sensitive element, its shape and size are limited to some extent. On the contrary, as in the thermistor thermometer the thermistor is a sensitive element as a whole, its shape and size are freely changeable. The thermistor is very convenient for obtaining a wide variety of shape, for example, bead, rod, disk, washer and flake types as well as the small size. Moreover, it is also easier to attach two or more sensitive elements to one thermometer. The thermistor thermometer is chosen, especially when a small one is in need.

Heat capacity of the thermometer is one of the factors which determine the time constant and the ability to follow the rapidly changing temperature. The smaller the size of the thermometer, the quicker it reaches

TABLE II

Intermittent Experiment

Time in minutes	Readings of the thermometers		
	Standard mercury thermometer °C	Thermistor thermometer °C	Platinum thermometer °C
0	22.4	22.40	22.40
1	23.0	23.00	22.95
2	25.0	24.95	24.90
3	26.1	26.10	26.10
4	27.0	27.05	27.05
4.5	28.2	28.15	28.20
5	29.1	29.10	29.10
6	29.8	29.80	29.80
6.5	30.9	30.85	30.80
7	31.7	31.70	31.65
7.5	32.6	32.55	32.60
8	33.5	33.45	33.50
9	34.5	34.45	34.50
10	35.8	35.75	35.80
10.5	36.8	36.75	36.80
11	37.5	37.50	37.50
11.5	38.5	38.50	38.50
12	39.2	39.15	39.20

TABLE III

Continuous Experiment

Time in minutes	Readings of the thermometers		
	Standard mercury thermometer °C	Thermistor thermometer °C	Platinum thermometer °C
0	36.5	36.50	36.50
1/3	35.9	35.90	35.95
2/3	35.3	35.35	35.30
1	34.3	34.30	34.30
1/3	33.7	33.70	33.70
2/3	32.4	32.45	32.50
2	31.6	31.60	31.60
1/3	30.6	30.65	30.60
2/3	29.6	29.65	29.60
3	28.9	28.85	28.90
1/3	27.9	27.90	27.95
2/3	27.1	27.10	27.15
4	26.3	26.25	26.30
1/3	25.3	25.35	25.30
2/3	25.0	25.00	24.95
5	24.6	24.60	24.55

the heat equivalence. The size of the thermometer depends upon both the sensitive element and its support. In this point, the thermistor thermometer is preferable to the platinum one.

In the present experiment the electrical resistance is much higher in the thermistor thermometer than in the platinum one. However, the ill-effect such as leakage in the tube, which disturbs the reading of the thermometer, was not seen. On the contrary, on account of its higher resistance value, the resistance of the lead wire does not become bothersome. Therefore, the use of a long lead wire is permissible.

The temperature coefficient of electrical resistance of the thermistor is much larger than that of the platinum, though the former is the negative coefficient contrary to the latter. The large value of temperature coefficient of the thermistor permits a new order of the sensitivity in thermometry. When the same order of the sensitivity is necessary, the thermistor thermometer is more accurate than the platinum one. In fact, our thermometer is very accurate.

The long-time stability of the thermistor thermometer is, according to the author's experience, almost the same as that of the platinum one. The stability of the thermistor thermometer, as development proceeds, may be expected to approach that of the precision platinum thermometer.

It is also favorable that the thermistor is inexpensive.

When the temperatures in not only two regions of the gastrointestinal tract but also more regions are investigated, more thermistors can be connected as mentioned above. Thus it is possible to measure simultaneously the temperatures in many regions of the gastrointestinal tract.

From the above discussion it is evident that the thermistor thermometer is a good one to measure the temperature in the gastrointestinal tract.

Summary

1. Using thermistors, a new thermometer to measure the temperatures in two or more regions of the gastrointestinal tract is described.
2. Comparison of this thermometer with the platinum one used previously was made.
3. It is proved that this thermometer is a good one to serve the purpose.

References

1) Masuda, Tohoku J. Exp. Med., 1951, **54**, 355.
2) Becker, Green & Pearson, Electr. Engineering, 1946, **65**, 711.
3) Forster & Ferguson, Amer. J. Physiol., 1952, **169**, 255.
4) Masuda, Ohara & Katsura, Tohoku Igaku Zassi, 1953, **47**-Supplement, 38.
5) Masuda, Tohoku J. Exp. Med., 1953, **57**, 119.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Diagnosis of Tumors by Needle Biopsy*

By

Kinshi Mitsuki
(參木 錦司)

From the Medical Clinic of Prof. T. Kurokawa,
Tohoku University, Sendai

(Received for publication, September 10, 1953)

The difficulties of the diagnosis and clinical differentiation of the various tumors, especially in upper abdominal region, are frequently encountered, however failure of multiple physical function tests to clarify many problems of this nature has been disappointing.

Since Lucatello,[1] Hellendal,[2] Martin-Ellis,[3] Stachel,[4] Iverson and Roholm[5] demonstrated that in properly selected patients, biopsy specimens could be obtained by aspiration through a needle with only a few unfavorable results, this procedure has helped to solve many difficult diagnostic problems by providing tissue containing several entire parenchyms of tissue for histo-pathological examination.

In recent years, needle biopsy findings have been employed by an increasing number of investigators as an aid in the diagnosis of pathology.

Many excellent reports on biopsy of the liver, including those of Rohom and others (1942),[6] Scherlock (1945),[7] Davis (1946),[8] Bierwaltes and Mallery (1946)[9], Hoffbauer (1947),[10] Kumpe (1947),[11] Volwiler and Jones (1947),[12] Franklin (1948),[13] Mateer and others (1948),[14] Buck (1948),[15] Popper (1949),[16] Schiff (1951),[17] Malcolm and their coworkers (1952)[18] have appeared in literature.

Having dealt with this problem, the author and his coworkers undertook a review of tissue biopsies done during the past fifteen years at Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai, Japan.

Methods

Construction of the Apparatus

The most importanct is attached to biopsy instruments designed to yield the maximum amount of tissue with a minimum amount of trauma. Hitherto the aspiration biopsy has been considered the simplest and

* Presented in the 35th General Meeting of the Gastro-enterological Society of Japan, Kyoto, April 1, 1949.

least traumatizing method but this is not always successful (Corriero,[19] Gillman and Gillman,[20] Nelson,[21] Silverman,[22] Hoffman[23]). To this end a new type of aspiration biopsy needle and method were devised by the author.

The instrument consists of an outer needle, with an internal diameter 2 mm., and 5 cm. in length, 20 cc. recordsyringe and so-called " Tissue-crushing apparatus."

Moreover this apparatus consists of two parts, the one is cannula of 12 cm. long and the other is an inner needle of 15 cm. which is split about 2 cm. longitudinally down to hub. The apex of this inner needle has a spoonlike or forklike structure.

The outer needle is inserted into the suspected tumor and then the cannula with split needle inserted closely in the bore of the outer needle.

The cannula is now advanced under the outer needle into the tumor for a distance of about $\frac{1}{2}$ cm. and pushed into the tumor mass cutting a tissue between its prong by manipulating of handles (Fig. 1, 2).

Fig. 1

Fig. 2

Fig. 1. So-called " Tissue-crushing apparatus".
Fig. 2. The apex of the apparatus.

Preparation of the patients

The patient is carefully selected according to physical conditions and kept in bed under close observation. The prothrombin time and bleeding time are determined routinely, as far as possible, in all patients and the relation between the tumor and surrounding tissues are investigated in aid of X-ray examination prior to the removal of specimen for biopsy.

No aspirations were done while the prothrombin concentration was less than 50 per cent of normal and it must be performed carefully in high fever, in combination of abscess, in hemorrhagic diathesis etc. Paracentesis preceded the biopsy in patients with ascites.

The needle puncture into tumor of surface of body is easier than that into deep one.

All biopsies for the abdominal tumors were generally performed directly to the tumor by abdominal approach, but in liver biopsies by the anterior subcostal approach in the medioclavicular line. When hepatic nodules were palpated the approach was varied in order to obtain a specimen from the nodular area.

Anesthesia

The skin over the suspected mass is thoroughly cleansed with antiseptic solution according to Grossich's method. 10 cc. of 1/2 per cent procaine hydrochloride are injected intradermally and subcutaneously over the site of puncture. As the needle approaches the tumor, the procaine should be squirted in under pressure.

The operator then pauses for 1 to 2 minutes so that the anesthetic takes effect before this sensitive region is punctured.

Introduction of the biopsy needle

After an interval of two to three minutes anesthesia is established. The operator usually works from the patient's right side.

On the abdominal approach, the patient is instructed to relax the abdominal muscles during the insertion of the needle. At first time the aspiration biopsy instrument is held at the junction of the needle and the syringe, and with the extended index finger supporting the needle at the site of entry through the skin.

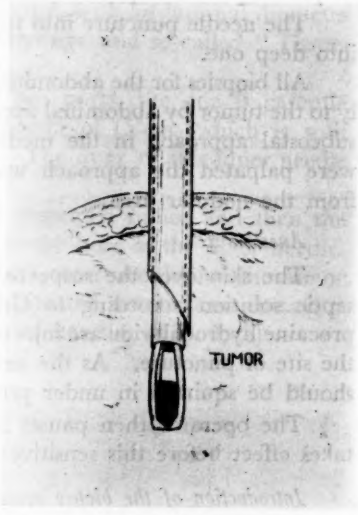
The needle is first gently pushed until the subcutaneous tissue is pierced, and then until the resistance of the surface of the subject is felt. The depth guard is then quickly adjusted so that the needle can be introduced only 2–3 cm. further. The needle is now plunged into the subject and at the same time, the syringe with needle is then quickly twisted on the plunger through 360 degrees and the plunger is quickly two or three times withdrawn.

By using suction of the syringe at various phase of the rotary course of the needle, more than ample materials are obtained for microscopic examination. And then in the skin puncture wound, 300,000 units of penicillin are injected and covered wtih a small cotton-wool moistened in alcohol. If tissue is not obtained the needle can, with safety, be introduced a second or even a third time. This, however, is rarely necessary. If

tissue is too hard to cut out, the so-called " Tissue-crushing apparatus " must be used (Fig. 3, 4).

Fig. 3

Fig. 4



Complication

The most serious potential complications of biopsy are generally considered to be those of hemorrhage, bile peritonitis due to puncture of a dilated aberrant bile duct, and infection.

Early reports have indicated a mortality of less than 1 or 2 per cent.

In our series the discomfort was felt by many patients when the trocar and cutting needle were introduced into the tissue. But, in one case in which needle biopsy had been done, blood was found in the ascitic fluid at the postmortem examination.

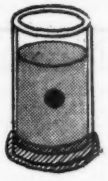
However, the cause of death was ascribed directly to a needle biopsy done for the differential diagnosis of jaundice. At the autopsy a bile peritonitis was found, apparently caused by the needle's entering an obstructed and dilated intrahepatic biliary duct producing oozing and bile peritonitis. In our experiences of biopsies, there has been no unfortunate case, except for above described one.

The making way of specimens

The needle biopsy offers the simplest method of obtaining material. On the other hand, the amount of tissue removed is smaller than that obtained by surgical means (a few cubic millimeters compared with cubic

centimeter). Therefore, there are many difficulties in making a microscopic specimen. According to this purpose, our new method is devised. The glass tube of about 0.5 cm. caliber, and 1.5 cm. length is prepared and the base of one side is closed with paraffin. The patient's venous blood is filled until the tube is half full. The small specimens are then laid in it, and then the blood is poured on it again. This glass tube containing specimen is placed after one hour in 30% formalin solution to prevent postmortem changes. A clotted blood removed from the tube after 24 hours is prepared for cutting in paraffin and staining in the usual manner (Fig. 5).

Fig. 5



Results

A review of our needle biopsies of the all tissues obtained in 253 cases, shows that the clinical diagnosis was confirmed in 216 cases or 85.4 per cent.

1) Liver biopsy

On the bases of our experience, we consider needle biopsy of the liver to be of clinical value in following seven categories:

1. Differential diagnosis of hepatic enlargement.
2. Differential diagnosis of jaundice and differentiation of extrahepatic and intrahepatic block.
3. Substantiating the presence of liver disease in patients with abnormal liver function tests.
4. Elucidation of the hepatosplenopathies.
5. Detection of neoplasm of the liver.
6. Evaluating and controlling therapy and estimating prognosis.
7. Following the course of the liver disease.

Since 1940, liver biopsies have been performed on 110 patients in our Clinic and the clinical diagnosis was confirmed in 101 cases or 91.8 per cent, however in six instances a second puncture and in one instance a third puncture was necessary to show the presence of tumor tissue. The clinical evaluation of liver biopsies is shown in Table I.

In 9 patients, the biopsy proved no aid in diagnosis, so actual percentage of failures based on 101 biopsies is 8.9 per cent.

Hepatic disease is often suspected when the liver is palpable in an individual giving a strong alcoholic history, but needle biopsy of the liver may reveal no abnormalities. Similarly, a palpable liver in a patient with a malignant tumor may wrongly suggest hepatic metastasis.

In the differentiation of medical and surgical jaundice, I have found liver biopsy of much greater value than a group of commonly employed liver fuction tests used individually or in combination. The biopsy

TABLE I

Clinical Evaluation of Liver Biopsy

(110 patients: 101 biopsies)

	No. of patients	Per cent
Clinical diagnosis confirmed	58	52.7
Clinical diagnosis corrected	28	25.4
Unsuspected disease detected	9	8.2
Total	95	86.3

diagnosis of obstructive jaundice is, in my experience, easily made after two weeks of jaundice.

We have been quite surprised to learn how often needle biopsy of the liver may reveal the presence of neoplasm.

It is of interest to note that a positive biopsy was relatively no more frequent in the cases in which the liver was palpated as nodular than in the cases in which it was palpated as smooth.

Looking at the diagnostic value of liver biopsy from another angle, it is seen from Table II that in 79 patients the confirmation of clinical diagnosis as result of biopsy was obtained.

TABLE II

Clinical diagnosis confirmed	No. of patients
Bile duct cancer	19
Metastatic cancer	13
Jaundice	10
Parenchymatous cancer	9
Spindle cell sarcoma	5
Congestive liver	5
Cirrhosis	4
Abscess	3
Degeneration	2
Tuberculosis	2
Acute yellow atrophy	1
Clonorchiasis hepatis	1
Syphilis	1
Hepatic glycogen disease	1
Perithelioma	1
Malaria	1
Kala azar	1

2) Tumors in abdominal region except liver tumors

Biopsies were performed in 27 cases of abdominal tumors. Diagnosis, which had been considered as doubtful possibilities, was substantiated in all cases. The clinically suspected diagnoses were vaguely abdominal tumors, but microscopically as follows: small spindle cell sarcoma, polymorph cell sarcoma, squamous epithelial cancer, round cell sarocma,

hypernephroma, giant cell sarcoma, tuberculoma, myxoma, hematoma, dermoid cyst, cylinderical epitherial cancer, pancreatic cancer, fibroma and peritoneal pseudomyxoma.

The needle biopsy of the abdominal tumors is valuable in the decisive diagnosis of the character of tumors, in evaluating and controlling therapy, and in estimating prognosis.

3) The lymph nodes

The difficulties of the diagnosis and clinical differentiation of various swelling of the lymph nodes are frequently encountered. Even the most adept diagnostician is often unable to define the exact diagnosis by ordinary methods of physical examination.

Should these nodules happen to be inflammatory in origin, or benign tumors, there is usually no urgency concerning proper recognition or treatment. On the other hand, should they represent a metastatic phase in early development of a malignant growth, then incorrect diagnosis and improper management may prove disastrous to the patient.

A leucemic node may often exactly simulate a lymphosarcoma, so that in an aleucemic leucemia a mistake may be inevitable. An innocent lymphoma may present an appearance identical with that of a lymphosarcoma or lymphogranuloma. The reticuloendothelial type may easily be mistaken for a secondary carcinoma. Moreover, it resembles to other condition, Hodgkin's disease and hyperplastic tuberculosis.

The exact diagnosis of lymph nodes is usually made by the microscopic examination of an excised superficial one.

But in the abscence of an exact diagnosis, or the ability to exclude a malignant growth, the needle biopsy for identification of the lesion is the only justifiable procedure.

Biopsies were performed in 51 cases with a swelling of lymph nodes and the confirmation of clinical diagnosis as result was obtained in Table III.

TABLE III

Clinical diagnosis confirmed	No. of patient
Metastases of carcinoma or sarcoma	21
Tuberculous lymphadenitis	12
Leucemia	4
Lymphosarcoma	4
Reticulosarcoma	3
Lymphogranuloma	3
Tularemia	2
Syphilis	1
Teratoma	1

4) The other organs

Needle biopsies of the other organs were often carried out, but our experiences on it were yet not large enough. Diagnoses which had been considered as doubtful possibilities were substantiated in following:

Sarcoma of great and small omentum, primary bronchogenic carcinoma of lung, hypernephroma, pseudomyxoma peritonei, neurofibroma of skin (Recklinghausen's disease), lymphangioma, mixed tumors and syphilis of parotid, carcinoma of rectum, carcinoma of pancreas, papillary carcinoma of urine bladder, malignant papillary cystadenoma of ovary, carcinoma of thyroid, lymphoid hyerplasia in colloid goitre, discovery of leishmania donovani and malaria plasmodium from spleen and differential diagnosis of hypertrophy or sarcoma of prostate etc.

Conclusion

1. At the Prof. T. Kurokawa's Clinic, needle biopsy of the various tumors, especially in abdominal cavity, has proved of great value in the five categories discussed. Namely:

a. Diagnosis which had been considered as doubtful possibilities was finally substantiated.

b. Decision of the character of malignant tumors.

c. Presumption of the original organ through the observation of metastatic region.

d. Evaluation and controlling therapy and estimating prognosis.

e. Following the course of the disease.

2. A review of our needle biopsies of the all tissues contained in 253 patients shows that the clinical diagnosis was confirmed in 216 cases or 85.4 per cent. Particularly, liver biopsies have been performed on 110 patients and positive pathologic findings obtained in 101 cases or 91.8 per cent.

3. The needle biopsy has gradually come to supplant the surgical biopsy because of its simplicity. When it is performed by physicians trained in the procedure, with the patients carefully selected, needle biopsy entails very little risk.

(The description of patient's histories and demonstration of the photographies in all cases were omitted owing to limited pages in this paper.)

I wish to express here my sincere thanks to Prof. T. Kurokawa who gave me encouragement and suggestions throughout the investigation and also I am grateful to the co-workers of our clinical department who have assisted in the studies on which these remarks are based: Dr. Y. Kurita, Dr. I. Aoyama, Dr. T. Sato and Dr. I. Amaki.

References

1) Lucatello, cited from Jahresbericht d. gesamt. Medizin, 1886, **11**, 23.

2) Hellendal, Z. kl. Med., 1899, **37**, 435.
3) Martin-Ellis, Ann. Surg., 1930, **92**, 169; Surg., Gynec. and Obst., 1934, **59**, 578.
4) Stachel, Schweiz. med. Wshr., 1939, 333.
5) Iverson & Roholm, Acta med. Scandinav., 1939, **102**, 1.
6) Roholm, Krarup & Iverson, Ergebn. d. inn. Med. u. Kinderh., 1942, **61**, 635.
7) Scherlock, Lancet, 1945, **2**, 397.
8) Davis, New Eng. J. Med., 1950, **243**, 2.
9) Bierwaltes & Mallery, Univ. Hosp. Bull. Ann Arbor, 1946, **12**, 13.
10) Hoffbauer, M. Clin. North America, 1945, **29**, 363.
11) Kumpe, Gastroenterology, 1947, **9**, 672.
12) Volwiler & Jones, New Eng. J. Med., 1947, **237**, 651.
13) Franklin, Popper, *et al.*, J. Lab. and Clin. Med., 1948, **33**, 435.
14) Mateer, Gastroenterology, 1948, **11**, 284.
15) Buck, J. Lab. and Clin. Med., 1948, **33**, 555.
16) Popper, Am. J. Med., 1949, **6**, 278; Proc. Central Soc. Clin. Research, 1946, **19**, 11.
17) Schiff, Ann. Int. Med., 1951, **34**, 948.
18) Malcolm & David, Gastroenterology, 1952, **21**, 245.
19) Corriero, Am. J. Surg., 1937, **37**, 341.
20) Gillman & Gillman, South African J. Med. Sc., 1945, **10**, 53.
21) Nelson, Gastroenterology, 1952, **21**, 289.
22) Silverman, Am. J. Surg., 1938, **40**, 671.
23) Hoffmann, Surg., Gynec. and Obst., 1933, **56**, 829.

The Tohoku Journal of Experimental Medicine, Vol. 60, No. 1, 1954

Studies on Mucopolysaccharide in Skin Diseases

II. Circumscribed Symmetrical Tuberous Myxedema*

By

Yoshiro Hamada
(濱田 芳郎)

From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai; Director: Prof. Minor Itô

(Received for publication, September 11, 1953)

Case Report

R. K., aged 34, a famer's wife came to our clinic on August 6, 1951, complaining of symmetrical swelling over face, both arms and legs.

Family history. Her father died at 62 years of age and her mother at 73 years. She is the seventh child. Three of eight brothers and sisters are still alive. She got married eleven years ago and has only three children in perfect health among eight pregnancies.

Past history. She has never suffered from notable disease except for removing of left axillar lymphglands in 1948.

Present illness. She had noticed swelling on face, both arms and legs since three years. Because of absence of any complains, she had not received medical treatment. The swelling increased its grade and distribution at the ninth day after the delivery of her eighth child on June 19, 1951. Moreover many subcutaneous tumors varying in size appeared on both arms and legs. Though complaining merely slight unpleasant feeling and stifling by walking, she was still working at home and farm.

Present status. She was well developed and well nourished. Body temperature was 36.1°C. Whistling heard at both pulmonary apex. No heart murmer. Puls 88. Blood pressure 110 mm. of mercury systolic and 80 diastolic. In the majority edema was found over the face especially on upper and lower eyelids and cheeks in bothside. Hair, eyebrows and eyelashes were normal. Swelling localized over both arms and legs especially over their anterolateral aspects respectively. Edematous skin covering these sites presented specific feature that it did not pit on pressure and felt firm, dry and cool. On the extensor surface of both arms plaques

* Read before the 114th Tohoku district meeting of Jap. Dermat. Assoc. Sendai, Nov. 23, 1951: and 19th Tohoku branch meeting of Jap. Soc. of Intern. Med., Sendai, Feb. 20, 1953.

resembling livedo marmorata purplish maroon in color were noticed. Slightly elevated about palm-sized lesions on the anterolateral surface of upper half of both legs showed more dilated and dimpling hair follicles and more lanugos than those of surroundings. Livedo marmoratas were found also over the area from lower half of both legs to both ankles but not so notable than those of arms. Numerous tumors varying in size from that of a rice-corn to that of a fingertip were palpable symmetrically and vertically on both pretibial regions. The consistence of these tumors was gelatinous and they felt as if one squeeses a sack of orange and they disappeared temporarily on pressure. No goiter, exophthalmos and tremor were present (Fig. 1 and 2).

Fig. 1

Fig. 2

Laboratory findings. No lymphadenitis was found all over the body. Mucous membrane of mouth normal. Red blood cell 4,600,000, Hb. 48% (Sahli), white blood cell 6500 (eosinophils 12%, neutrophils 97%, lymphocyte 6% and monocyte 6%). Blood Wassermann, Kahn and Murata negative. Aschner's phenomenon positive. Sensitive to epinephrine but not so sensitive to pilocarpine. Diaphoretic test (Prof. Wada's[1] Method) 10^{-6}. Normal fundus oculi. Hyperemic tonsilla pallatina and anemic vocal cords bothside. B.M.R., plus 10% (Read's formula) Grip power 18 kg. (left), 22 kg (right). Blood sugar 65 mg/dl. Thorn's test —28.7%; Uric acid 45.9 mg/dl, creatinine 72.7 mg/dl before epinephrine injection and 66.7 mg/dl, 100 mg/dl respectively after injection and increasing in uric-acid/creatinine ratio was plus 6.3%. Roentgenogram of

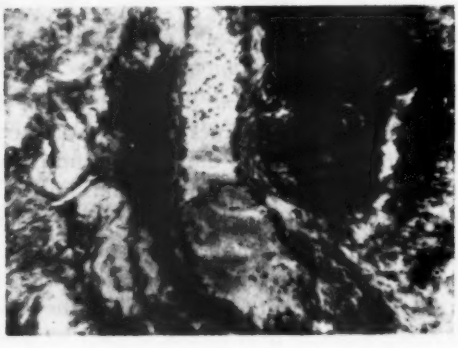
sella turcica showed 10.0 mm. in distance from Tuberculum sellae to Dorsum sellae, 8.8 mm. in depth and 64.0 mm² in lateral area. Thyroid stimulating hormone in blood was estimated by De Robertis method and cytological coefficient was 83. (Dr. T. Okada, of Prof. Katsura's clinic of surgery, Tohoku University).

Histological findings. Biopsy was made on the extensor aspect of left arm, and a tumor of finger-tip in size was also removed.

SKIN. Stratum corneum was slight thickened. Stratum granulosum consisted of one or two layers in general, but hypertrophied in three or four layers at the site of immediatly underneath papillae dermis presented marked mucinous degeneration. Prickle cell layer was normal in depth but perinuclear vacuole formation was found here and there. Basal layer showed palisade arrangement of cylindric cells and slight hyperpigmentation. In general epidermal processes were somewhat irregular in their arrangement. Papillae dermis and subpapillary layer became homogeneous. Their capillary endothel showed extreme hypertrophy and poor staining. Some of the papillae were so spongy and poorly-staining with mucicarmine that the common connective tissue structure was lacking. In pars reticularis the connective tissue fibers became irregular and dilated blood vessels were noticed. These vessel endothel cells were hypertrophied and surrounded by few fibroblasts, small round cells and mast cells. Connective tissue became homogeneous. No alterations were found in sweat duct, hair follicle, arector pili muscle but mucicarmine staining halo (M)

Fig. 3

Fig. 4



resembling livedo marmorata purplish maroon in color were noticed. Slightly elevated about palm-sized lesions on the anterolateral surface of upper half of both legs showed more dilated and dimpling hair follicles and more lanugos than those of surroundings. Livedo marmoratas were found also over the area from lower half of both legs to both ankles but not so notable than those of arms. Numerous tumors varying in size from that of a rice-corn to that of a fingertip were palpable symmetrically and vertically on both pretibial regions. The consistence of these tumors was gelatinous and they felt as if one squeeses a sack of orange and they disappeared temporarily on pressure. No goiter, exophthalmos and tremor were present (Fig. 1 and 2).

Fig. 1

Fig. 2

Laboratory findings. No lymphadenitis was found all over the body. Mucous membrane of mouth normal. Red blood cell 4,600,000, Hb. 48% (Sahli), white blood cell 6500 (eosinophils 12%, neutrophils 97%, lymphocyte 6% and monocyte 6%). Blood Wassermann, Kahn and Murata negative. Aschner's phenomenon positive. Sensitive to epinephrine but not so sensitive to pilocarpine. Diaphoretic test (Prof. Wada's[1] Method) 10^{-5}. Normal fundus oculi. Hyperemic tonsilla pallatina and anemic vocal cords bothside. B.M.R., plus 10% (Read's formula) Grip power 18 kg. (left), 22 kg (right). Blood sugar 65 mg/dl. Thorn's test —28.7%; Uric acid 45.9 mg/dl, creatinine 72.7 mg/dl before epinephrine injection and 66.7 mg/dl, 100 mg/dl respectively after injection and increasing in uric-acid/creatinine ratio was plus 6.3%. Roentgenogram of

sella turcica showed 10.0 mm. in distance from Tuberculum sellae to Dorsum sellae, 8.8 mm. in depth and 64.0 mm^2 in lateral area. Thyroid stimulating hormone in blood was estimated by De Robertis method and cytological coefficient was 83. (Dr. T. Okada, of Prof. Katsura's clinic of surgery, Tohoku University).

Histological findings. Biopsy was made on the extensor aspect of left arm, and a tumor of finger-tip in size was also removed.

SKIN. Stratum corneum was slight thickened. Stratum granulosum consisted of one or two layers in general, but hypertrophied in three or four layers at the site of immediatly underneath papillae dermis presented marked mucinous degeneration. Prickle cell layer was normal in depth but perinuclear vacuole formation was found here and there. Basal layer showed palisade arrangement of cylindric cells and slight hyperpigmentation. In general epidermal processes were somewhat irregular in their arrangement. Papillae dermis and subpapillary layer became homogeneous. Their capillary endothel showed extreme hypertrophy and poor staining. Some of the papillae were so spongy and poorly-staining with mucicarmine that the common connective tissue structure was lacking. In pars reticularis the connective tissue fibers became irregular and dilated blood vessels were noticed. These vessel endothel cells were hypertrophied and surrounded by few fibroblasts, small round cells and mast cells. Connective tissue became homogeneous. No alterations were found in sweat duct, hair follicle, arector pili muscle but mucicarmine staining halo (M)

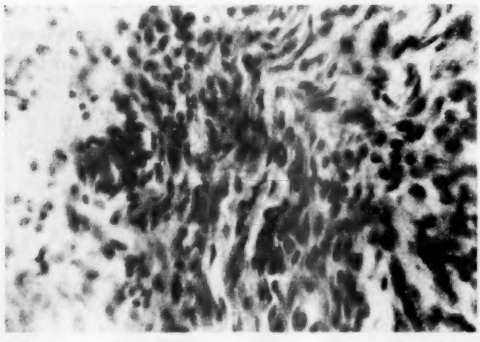
Fig. 3

Fig. 4

was found arround sweat gland (Fig. 3).

SUBCUTANEOUS TUMOR. At the first glance the picture was that of fresh granuloma. Blood vessels were noticed as the mantling around newly formed tissue. Perilymphatic-vessel infiltration was suggested according to the presence of intense collaret-like small round cell infiltration about the blood vessel walls. These cell infiltration consisted from plasma cells, mast cells and reticular network of connective tissue rich in fibroblasts (Fig. 4). Some of dilated blood vessel was filled with vast blood cells. Hypertrophy of vessel endothel cells and narrowness of vessel cavity were noticed. Polynuclear leucocytes and big monocyte-like cells scattered amidst homogeneous connective tissue (Fig. 5). Lison's staining revealed marked proliferation of mast cells surrounded by connective tissue network. Thus it is reasonable that these substances were palpable clinically as a tumor. The pattern gives us the impression of fresh elephantiasis which is originated from the degenerative changes of blood- and lymphatic-vessel wall. Metachromasia also noticed in connective tissue.

Fig. 5



Course of treatment. On August 6, 1951 she was given dessicated thyroid 0.1 g. three times a day. Administration was continued thereafter in increasing dose to daily 0.6 g. until september 25, 1951. Prominent decreasing of edema and diminishing of tumors was noticed after the total dose of 8.0 g. She was then placed under treatment with daily 100 g. of 1% pottasium iodide solution by mouth for three weeks. Edema got worse. Therefore dessicated thyroid was given again. Besides thyroid treatment, according to the histological findings especially of Lison's staining hyaluronidase (500 V. U. M.) in total was injected in the tumors over right arm and right leg. The effect of hyaluronidase injection was noticed macroscopically by the revolution of tumors but the

biopsy could not be performed because the patient did not visit the clinic again.

Comment

Since Millard described the concomitant edema in a case of hypothyroidism, the existence of a peculiar type myxedema in hyper- or euthyroid states called the attention in the field of internal and dermatological investigators. Sollier was the first to note such case. Dubreuilh[2] termed it fibromes miliaires folliculaire, Lewtschenkoff proposed the term dermatomyxom and Reitmann termed atypical scleroderma. In 1916 Jadassohn-Dösseker[3] observed histologically mucin in the corium in a 51 years male who had elastic edema of face and upper extremities, tubercles on scrotum, hypertrophied mucous membrane of mouth, tumors over neck and shoulder, and classified their case as a atypical myxedema. Keining[4] in 1928 reported bilateral circumscribed myxedema in two female patients of Grave's disease and in the discussion Jadassohn emphasized the importance in the histological investigation of mucin and the idea of individual specific inclination to mucinous infiltration was stressed and Pulvermacher also noticed unsucseptibility of certain peripheral region against thyroid homrone according to the similar feature of the predilection of localized myxedema to that of drug eruption. Keining concluded the pathogenesis as the mixed type of hyper- and hypothyroidism and used the term abortive tuberous myxedema. Cohen also proposed thyrotoxic circumscribed myxedema.

The reported cases amount about 100 in total. Most of them showed goiter in their past histories or present status and the cutaneous lesions develop before or after the reappearance of toxic state following total or subtotal thyroidectomia as showed by Trotter-Eden's symmary of 68 cases, i.e. 32 cases before various treatment of hyperthyroidic state, 2 cases after its X-ray treatment, 35 cases after subtotal thyroidectomy and they found 3% circumscribed myxedema among 134 thyrotoxicosis. French[5] said that localized myxedema in euthryroid state such as reported by Bloom and other investigators is found asymmetrically on the upper or lower extremities or on the trunk in contrast with the symmetrical distribution in hyperthyroid state. Complication of progressive exophthalmos with circumscribed myxedema especially in the majority of plaque type was reported by Curtis,[6] Pillsbury and Stokes[7] but its total cases are about ten in all until 1949.

The author's analysis of 66 cases reported in the literatures until 1953 in spite of many lacks in back-numbers is as follows: 26 males, 34 females, 6 cases sex is not given; among 34 females, 9 married, 3 not married and 22 cases not given; Morrow's[8] case is the only one which

became manifest after delivery.

On the pathogenesis of the condition we have no definite conclusion as yet and many investigators discussed about socalled polyglandular dysharmonies. Bogroff noticed the enlarged sella turcica in Goldenberg's case, Richter[9] found hyperplastic thymus in a necropsy and he emphasized the generalized dysfunction of whole internal secretion. Recently Grynkewich[10] reported the response of pituitary to epinephrine measuring a fall in circulating eosinophiles. Fischer guessed primary desequilibrium in neurohormonal system according to the hyperalbuminemia in 49 years female and Vilanova stressed the hypersecretion of pituitary thyrotropic homone in a case complicated with exophthalmos.

On the origin of diffuse infiltration of mucinous substance Watson[16] found the nature to be the compound of hyaluronic acid and chondoroitin or mucoitin sulfuric acid. Bloom *et al.*, Grais, Asboe-Hansen,[12] Watrin[13] have used hyaluronidase in the treatment of localized myxedema. Recently Gottron[14] conducted also histoenzymological study on a 49 years male but he emphasized the reduced blood flow as a important cause according to the reducing of photosensitivity and skin temperature and prolonged Q. R. Z.

Conclusion

1. Circumscribed symmetrical tuberous myxedema in a female who had never suffered from goiter or exophthalmos and got worse by delivery was reported.
2. Proliferation of mast cell and homogeneous connective tissue were found by Lison's staining.
3. Polyglandular dysharmony was evident from increased pituitary thyroid stimulating hormone in blood, eosinophilic response following epinephrine injection and small sella turcica.
4. Clinical improvement was noticed with the use of dessicated thyroid and hyaluronidase.

References

1) Wada, Tohoku J. Exp. Med., 1948, **49**, 284.
2) Dubreuilh, Bull. Soc. Franç. Dermat., 1921, **28**, 221.
3) Jadassohn-Dösseker, Arch. f. Dermat., 1916, **123**, 177.
4) Keining, Dermat. Wschr., 1928, **87**, 1874.
5) French, New. Eng. J. Med., 1949, **241**, 299.
6) Curtis *et al.*, Arch. of Dermat., 1949, **60**, 318.
7) Pillsbury & Stokes, Arch. of Dermat., 1931, **24**, 255.
8) Morrow, Brit. J. Dermat., 1899, **11**, 286.
9) Richter, Dermat. Wschr., 1927, **84**, 51.
10) Grynkewich *et al.*, Amer. J. Med. Sci., 1943, **222**, 142.

11) Watson, Canad. Med. Asoc. J., 1946, **54**, 261.
12) Asboe-Hansen, J. Invest. Dermat., 1950, **15**, 25.
13) Watrin, Ann. dermat. syph., 1951, **78**, 23.
14) Gottron & Korting, Arch. f. Dermat., 1953, **195**, 625.